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FILE 'MEDLINE' ENTERED AT 12:59:11 ON 01 MAY 2003

=> d que l10
L1          579 SEA ("BARRY M"/AU OR "BARRY M A"/AU)
L2          108 SEA ("BARRY MICHAEL"/AU OR "BARRY MICHAEL A"/AU OR "BARRY
                MICHAEL ALAN"/AU)
L4          687 SEA L1 OR L2
L5          517 DUP REM L4 (170 DUPLICATES REMOVED)
L6         3107 SEA SHERMANII OR PROPRION? OR TRANSCARBOXYLAS?
L7            2 SEA L5 AND L6
L8           10 SEA L5 AND BIOTIN?
L9            8 SEA L5 AND (CHIMER? OR FUSION)
L10          16 SEA L7 OR L8 OR L9


=> d bib ab it 1-16

L10  ANSWER 1 OF 16  HCAPLUS  COPYRIGHT 2003 ACS
AN   2003:179368  HCAPLUS
TI   Metabolic biotinylation of proteins and viral vectors for
     purification from mammalian cells
AU   Barry, Michael A.; Parrott, M. Brandon; Adams, Kristen E.;
     Campos, Samuel; Ghosh, Debadyuti
CS   Center for Cell and Gene Therapy and Department of Bioengineering, Baylor
     College of Medicine and Rice University, Houston, TX, 77030, USA
SO   Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United
     States, March 23-27, 2003 (2003), BIOT-376 Publisher: American Chemical
     Society, Washington, D. C.
     CODEN: 69DSA4
DT   Conference; Meeting Abstract
LA   English
AB   We have previously demonstrated the ability to metabolically biotinylate
     genetically-tagged proteins in mammalian cells and in living mice.  To
     extend this technol. for gene therapy vectors, the adenoviral fiber was
     genetically engineered for metabolic biotinylation to generate a vector
     that is covalently biotinylated by the holocarboxylase synthase enzyme of
     293 virus producer cells.  In addn. to re-targeting applications, we
     demonstrate the ability to magnetically sep. the biotinylated virus as
     well as affinity purify it on monomeric avidin using biotin as a
     biocompatible release agent.  This work on adenovirus provides proof of
     principle for a unified vector technol. for vector purifn. on monomeric
     avidin (Kd=10-7 M) and vector re-targeting by conjugation of biotinylated
     ligands to the vector using tetrameric avidin (Kd up to 10-15 M).
     Generation of metabolically biotinylated VSV-g proteins and AAV vectors
     provides addnl. proof of principle for this approach for variety of viral
     gene therapy vectors.

L10  ANSWER 2 OF 16  HCAPLUS  COPYRIGHT 2003 ACS
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AN 2003:179348 HCAPLUS
TI Cell targeting using metabolically **biotinylated** adenoviral vectors
AU **Barry, Michael A.**; Parrott, M. Brandon; Campos, Samuel; Adams, Kristen E.
CS Center for Cell and Gene Therapy and Department of Bioengineering, Baylor College of Medicine and Rice University, Houston, TX, 77030, USA
SO Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003 (2003), BIOT-356 Publisher: American Chemical Society, Washington, D. C.
CODEN: 69DSA4
DT Conference; Meeting Abstract
LA English
AB Current gene therapy vectors are limited by an inability to deliver therapeutic genes specifically to target cells. For viral vectors, one method of altering vector specificity is to genetically introduce cell-targeting ligands into the structure of the viral fiber protein. For many ligands, these insertions either disrupts the function of the ligand or reciprocally ablates the function of the virus. As one approach to address this problem, the adenoviral fiber was genetically engineered to be metabolically biotinylated in mammalian cells to generate a vector directly from virus producer cells that could be purified on monomeric avidin (Kd=10-7 M) and be re-targeted to alternate receptors by conjugation of biotinylated ligands to the virus using tetrameric avidin (Kd up to 10-15 M). We demonstrate here the ability to efficiently re-target cells by conjugation of the virus to biotinylated antibodies both in vitro and in vivo.

L10 ANSWER 3 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 2002:755073 HCAPLUS
DN 137:275370
TI Methods for the in vivo **biotin** labeling of polypeptides
IN **Barry, Michael A.**; Parrott, Michael B.
PA Baylor College of Medicine, USA
SO U.S. Pat. Appl. Publ., 13 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2002142355	A1	20021003	US 2001-987485	20011114
PRAI US 2000-247965P	P	20001114		

AB The present invention is directed to methods that can used for biotin labeling polypeptides in mammalian cells. The methods can be effectively used for cytoplasmic proteins, secreted proteins, and for proteins found on viral surfaces.
IT Peptides, biological studies
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(**Biotin** acceptor; methods for in vivo **biotin** labeling of polypeptides)
IT Animal cell line
(CHO; methods for in vivo **biotin** labeling of polypeptides)
IT Proteins
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(Cell Surface; methods for in vivo **biotin** labeling of polypeptides)
IT Proteins

RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(Secreted; methods for in vivo **biotin** labeling of polypeptides)

IT Proteins
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(Viral surface; methods for in vivo **biotin** labeling of polypeptides)

IT Gene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(expression; methods for in vivo **biotin** labeling of polypeptides)

IT Proteins
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(fiber; methods for in vivo **biotin** labeling of polypeptides)

IT Cell
(host; methods for in vivo **biotin** labeling of polypeptides)

IT Immunoassay
(immunoblotting; methods for in vivo **biotin** labeling of polypeptides)

IT Immunoassay
(immunohistochem.; methods for in vivo **biotin** labeling of polypeptides)

IT Animal cell
(mammalian; methods for in vivo **biotin** labeling of polypeptides)

IT Adenoviridae
Animal tissue culture
Biotinylation
Cytoplasm
Gel electrophoresis
Genetic code
Genetic engineering
Genetic vectors
Interface
Retroviridae
Transformation, genetic
Virus
(methods for in vivo **biotin** labeling of polypeptides)

IT Amino acids, biological studies
Nucleotides, biological studies
Polynucleotides
Promoter (genetic element)
Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methods for in vivo **biotin** labeling of polypeptides)

IT **Fusion** proteins (**chimeric** proteins)
Peptides, biological studies
Proteins
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(methods for in vivo **biotin** labeling of polypeptides)

IT Avidins
Ligands
RL: RCT (Reactant); RACT (Reactant or reagent)
(methods for in vivo **biotin** labeling of polypeptides)

IT 39419-81-3, **Biotin** ligase
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(BirA; methods for in vivo **biotin** labeling of polypeptides)
IT 466701-20-2
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(amino acid sequence; methods for in vivo **biotin** labeling of polypeptides)
IT 56-87-1, Lysine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methods for in vivo **biotin** labeling of polypeptides)
IT 58-85-5, **Biotin** 9014-19-1, Pyruvate carboxylase 9023-93-2, Acetyl CoA carboxylase 9023-94-3, Propionyl-CoA carboxylase 9023-95-4, Methylcrotonyl-CoA carboxylase
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(methods for in vivo **biotin** labeling of polypeptides)
IT 466712-47-0 466712-48-1
RL: PRP (Properties)
(unclaimed nucleotide sequence; methods for the in vivo **biotin** labeling of polypeptides)
IT 466712-46-9
RL: PRP (Properties)
(unclaimed protein sequence; methods for the in vivo **biotin** labeling of polypeptides)
IT 225371-20-0 466712-49-2 466712-50-5
RL: PRP (Properties)
(unclaimed sequence; methods for the in vivo **biotin** labeling of polypeptides)

L10 ANSWER 4 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 2002:199263 HCAPLUS
DN 136:308125
TI **Fusion** protein vectors to increase protein production and evaluate the immunogenicity of genetic vaccines
AU Wu, Lei; **Barry, Michael A.**
CS Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, 77030, USA
SO Molecular Therapy (2000), 2(3), 288-297
CODEN: MTOHCK; ISSN: 1525-0016
PB Academic Press
DT Journal
LA English
AB Genetic immunization is a method for vaccination and lab. antibody prodn. where antigen-expressing plasmids are introduced into animals to elicit immune responses. Although genetic immunization works well for many antigens, problems can arise with protein sequences that (i) are toxic to host cells, (ii) are difficult to translate by mammalian cells, or (iii) evade immune presentation. The authors demonstrate here the ability to increase protein prodn. and antigen secretion by the simple method of fusing poorly expressed sequences to well-expressed heterologous proteins. Proof-of-principle is demonstrated here using the poorly translated HIV-1 envelope whose protein prodn. is rescued by fusing this antigen to the carboxy-termini of two well-expressed proteins: the cytoplasmic green fluorescent protein and the secreted human protein .alpha.1-antitrypsin. This approach represents a simple and substantially less expensive method to increase protein and antigen prodn. than codon-optimization strategies. It may therefore be more useful than whole gene codon replacement to enable inexpensive lab. antibody prodn. of poorly expressed antigens and for large-scale genomic protein or antigen screening efforts. Finally, the authors demonstrate a second benefit of this antigen fusion strategy in which the test antigen is "sandwiched" between two pos. control

antigens. By this approach, the authors demonstrate the intrinsic lack of immunogenicity of HIV-1 envelope under conditions when robust antibody responses are generated against its fusion protein partners, but not against this evasive antigen. These fusion protein vectors therefore represent a simple approach to not only increase antigen prodn., but also assess antigen prodn. and immunogenicity in vivo. (c) 2000 Academic Press.

IT Vaccines
(AIDS; antigen **fusion** proteins for increased expression by genetic vaccines)

IT Human immunodeficiency virus 1
Plasmid vectors
(antigen **fusion** proteins for increased expression by genetic vaccines)

IT Antigens
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**fusion** products, with carrier proteins; for increased expression by genetic vaccines)

IT Immunization
(genetic; antigen **fusion** proteins for increased expression by genetic vaccines)

IT Envelope proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gp120env, **fusion** products, with carrier proteins; for increased expression by genetic vaccines)

IT Envelope proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gp140env, **fusion** products, with carrier proteins; for increased expression by genetic vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(green fluorescent, **fusion** products, with antigens; for increased expression by genetic vaccines)

IT **Fusion** proteins (**chimeric** proteins)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(of protein antigens for increased expression by genetic vaccines)

IT Secretion (process)
(protein; antigen **fusion** proteins for increased expression by genetic vaccines)

IT Vaccines
(synthetic; antigen **fusion** proteins for increased expression by genetic vaccines)

IT Anti-AIDS agents
(vaccines; antigen **fusion** proteins for increased expression by genetic vaccines)

IT 9041-92-3D, .alpha.1-Antitrypsin, protein antigen **fusion** products
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(for increased expression by genetic vaccines)

RE.CNT 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 2002:199169 HCAPLUS
DN 137:75477
TI Metabolic **biotinylation** of recombinant proteins in mammalian cells and in mice
AU Parrott, M. Brandon; **Barry, Michael A.**
CS Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX, 77030, USA

SO Molecular Therapy (2000), 1(1), 96-104
CODEN: MTOHCK; ISSN: 1525-0016
PB Academic Press
DT Journal
LA English
AB The avidin-biotin system is a fundamental technol. in biomedicine for immunolocalization, imaging, nucleic acid blotting, and protein labeling. While this technol. is robust, it is limited by the fact that mammalian proteins must be expressed and purified prior to chem. biotinylation using crosslinking agents which modify proteins at random locations to heterogeneous levels and can inactivate protein function. To circumvent this limitation, we demonstrate the ability to metabolically biotinylate tagged proteins in mammalian cells and in mice using the endogenous biotinylation enzymes of the host. Endogenously biotinylated proteins were readily purified from mammalian cells using monomeric avidin and eluted under nondenaturing conditions using only biotin as the releasing agent. This technol. should allow recombinant proteins and fragile protein complexes to be produced and purified from mammalian cells as well as from transgenic plants and animals. In addn., this technol. may be particularly useful for cell-targeting applications in which proteins or viral gene therapy vectors can be biotinylated at genetically defined sites for combination with other targeting moieties complexed with avidin. (c) 2000 Academic Press.
IT Post-translational processing
(**biotinylation**; metabolic **biotinylation** and purifn. of recombinant proteins fused to P. **shermanii transcarboxylase** domain in mammalian cells and mice)
IT Cricetulus griseus
Mouse
Propionibacterium **shermanii**
Protein motifs
(metabolic **biotinylation** and purifn. of recombinant proteins fused to P. **shermanii transcarboxylase** domain in mammalian cells and mice)
IT **Fusion** proteins (**chimeric** proteins)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(metabolic **biotinylation** and purifn. of recombinant proteins fused to P. **shermanii transcarboxylase** domain in mammalian cells and mice)
IT Protein sequences
(of **transcarboxylase**; metabolic **biotinylation** and purifn. of recombinant proteins fused to P. **shermanii transcarboxylase** domain in mammalian cells and mice)
IT 58-85-5, **Biotin**
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(metabolic **biotinylation** and purifn. of recombinant proteins fused to P. **shermanii transcarboxylase** domain in mammalian cells and mice)
IT 9029-86-1, **Transcarboxylase**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(metabolic **biotinylation** and purifn. of recombinant proteins fused to P. **shermanii transcarboxylase** domain in mammalian cells and mice)
RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 6 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 2002:12604 HCAPLUS

DN 136:198537
TI Generation of genome-wide CD8 T cell responses in HLA-A*0201 transgenic mice by an HIV-1 ubiquitin expression library immunization vaccine
AU Singh, Rana A. K.; Wu, Lei; **Barry, Michael A.**
CS Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, 77030, USA
SO Journal of Immunology (2002), 168(1), 379-391
CODEN: JOIMA3; ISSN: 0022-1767
PB American Association of Immunologists
DT Journal
LA English
AB HIV-1 is a fundamentally difficult target for vaccines due to its high mutation rate and its repertoire of immunoevasive strategies. To address these difficulties, a multivalent, proteasome-targeted, live genetic vaccine was recently developed against HIV-1 using the expression library immunization approach. In this HIV-1 vaccine all open reading frames of HIV-1 are expressed from 32 plasmids as Ag fragments fused to the ubiquitin protein to increase Ag targeting to the proteasome to enhance CTL responses. In this work the authors demonstrate the ability of the HIV-1 library vaccine to simultaneously provoke robust HLA-A*0201-restricted T cell responses against all 32 HIV-1 library vaccine Ags after single immunization by gene gun. These CD8 T cell responses included HLA-A*0201-restricted CTL activity, CD8/IFN-.gamma. T cell responses, and HLA tetramer binding against defined immunodominant epitopes in gag, pol, env, and nef as well as potent CD8/IFN-.gamma. responses against undefined HLA-A*0201-restricted epitopes in all remaining Ags of the library. CD8 responses mediated by single gag, pol, env, and nef plasmids from the vaccine demonstrated little redn. in specific T cell responses when these plasmids were dild. into the context of the full 32-plasmid library, suggesting that Ag dominance or immune interference is not an overt problem to limit the efficacy of this complex vaccine. Therefore, this work demonstrates the ability of the HIV-1 library vaccine to generate robust multivalent genome-wide T cell responses as one approach to control the highly mutable and immunoevasive HIV-1 virus.
IT Vaccines
(AIDS; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on ubiquitin expression library immunization in relation to)
IT Human immunodeficiency virus 1
Plasmid vectors
(HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on ubiquitin expression library immunization)
IT Histocompatibility antigens
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(HLA-A2.1; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on ubiquitin expression library immunization)
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ORF, **fusion** products with ubiquitin; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on immunization with)
IT T cell (lymphocyte)
(cytotoxic; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on ubiquitin expression library immunization)
IT gag proteins
nef protein
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**fusion** products, with ubiquitin; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on immunization with)

IT Envelope proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gene env, **fusion** products, with ubiquitin; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on immunization with)
IT Enzymes, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gene pol, **fusion** products, with ubiquitin; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on immunization with)
IT Immunization
(genetic; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on ubiquitin expression library immunization)
IT Anti-AIDS agents
(vaccines; HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on ubiquitin expression library immunization in relation to)
IT 60267-61-0D, Ubiquitin, **fusion** products with HIV-1 antigens
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(HLA-A2-restricted cytotoxic T-cell response to human immunodeficiency virus on immunization with)
RE.CNT 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 7 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 2001:164286 HCAPLUS
DN 134:292368
TI Metabolic **biotinylation** of secreted and cell surface proteins from mammalian cells
AU Parrott, M. Brandon; **Barry, Michael A.**
CS Department of Immunology, Baylor College of Medicine, Houston, TX, USA
SO Biochemical and Biophysical Research Communications (2001), 281(4), 993-1000
CODEN: BBRCA9; ISSN: 0006-291X
PB Academic Press
DT Journal
LA English
AB Due to its strength and specificity, the interaction between avidin and biotin has been used in a variety of medical and scientific applications ranging from drug targeting to immunohistochem. To maximize the application of this technol. in mammalian systems, we recently demonstrated the ability to metabolically biotinylate tagged proteins in mammalian cells using the endogenous biotin ligase enzymes of the mammalian cell. This technol. allows site-specific biotinylation without any exogenous reagents and eliminates possible inactivation of the protein of interest by nonspecific biotinylation. Here, we report further expansion of the mammalian metabolic biotinylation technol. to enable biotinylation of proteins secreted from mammalian cells and expressed on their cell surface by cosecretion with BirA, the biotin ligase of E. coli. This technique can be used to biotinylate secreted proteins for purifn. or targeting and also for biotinylating the surfaces of mammalian cells to facilitate their labeling and purifn. from other nontagged cells. (c) 2001 Academic Press.
IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(green fluorescent; metabolic **biotinylation** of secreted and cell surface proteins from mammalian cells)
IT Animal cell
(mammalian; metabolic **biotinylation** of secreted and cell

surface proteins from mammalian cells)
IT **Biotinylation**
Cell membrane
Propionibacterium **shermanii**
(metabolic **biotinylation** of secreted and cell surface proteins from mammalian cells)
IT Proteins, general, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(metabolic **biotinylation** of secreted and cell surface proteins from mammalian cells)
IT 9003-99-0, Peroxidase
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(horseradish; metabolic **biotinylation** of secreted and cell surface proteins from mammalian cells)
IT 9035-81-8, Antitrypsin 9067-78-1, **Transcarboxylase**
39419-81-3, **Biotin** ligase
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(metabolic **biotinylation** of secreted and cell surface proteins from mammalian cells)
RE.CNT 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 8 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 1996:718327 HCAPLUS
DN 126:6443
TI Expression library immunization
IN Johnston, Stephen A.; **Barry, Michael A.**; Lai, Wayne C.
PA Board of Regents, the University of Texas System, USA
SO PCT Int. Appl., 100 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9631613	A1	19961010	WO 1996-US4561	19960403
	W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML				
	US 5703057	A	19971230	US 1995-421155	19950407
	CA 2216092	AA	19961010	CA 1996-2216092	19960403
	AU 9653843	A1	19961023	AU 1996-53843	19960403
	AU 717091	B2	20000316		
	EP 819177	A1	19980121	EP 1996-910725	19960403
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI				
	JP 11503607	T2	19990330	JP 1996-530449	19960403
	US 5989553	A	19991123	US 1997-1157	19971230
PRAI	US 1995-421155		19950407		
	WO 1996-US4561		19960403		

AB A general method for vaccinating against any pathogen is presented. The method utilizes expression library immunization, where an animal is inoculated with an expression library constructed from fragmented genomic DNA of the pathogen. All potential epitopes of the pathogen's proteins

are encoded in its DNA, and genetic immunization is used to directly introduce one or more expression library clones to the immune system, producing an immune response to the encoded protein. Inoculation of expression libraries representing portions of the Mycoplasma pulmonis genome, was shown to protect mice from subsequent challenge by this natural pathogen. Protection against Listeria was also obtained using the method.

IT Vaccines
(expression library immunization)

IT DNA
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(expression library of genomic; expression library immunization)

IT cDNA
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(expression library of; expression library immunization)

IT Animal cell
Escherichia coli
(mammalian, expression library cloning in; expression library immunization)

IT Antibodies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(to expression library epitopes; expression library immunization)

IT Algae
Animal virus
Bacteria (Eubacteria)
Listeria monocytogenes
Mold (fungus)
Mycoplasma pulmonis
Neoplasm
Pathogen
Protozoa
Yeast
(vaccines for; expression library immunization)

IT 9075-08-5, Nuclease, restriction endodeoxyribo-
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(DNA fragmentation by; expression library immunization)

IT 12629-01-5DP, Somatotropin (human), **fusion** proteins with
60267-61-0DP, Ubiquitin, **fusion** proteins with
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(expression library immunization)

L10 ANSWER 9 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 1996:145751 HCAPLUS
DN 124:280452
TI Toward cell-targeting gene therapy vectors: selection of cell-binding peptides from random peptide-presenting phage libraries
AU **Barry, Michael A.**; Dower, William J.; Johnston, Stephen Albert
CS Dep. Int. Med., Univ. Texas Southwest. Med. Cent., Dallas, TX, 75235-8573, USA
SO Nature Medicine (New York) (1996), 2(3), 299-305
CODEN: NAMEFI; ISSN: 1078-8956
PB Nature Publishing Co.
DT Journal
LA English
AB Ideal gene therapy vectors would be delivered i.v. to transfect only specific cells. Existing vectors only transfect cells in vivo in a manner detd. by blood flow and the site of introduction. As a general and systematic approach for generating cell-targeting ligands for gene therapy

vectors, we have used peptide-presenting phage libraries to select peptides that bind and enter several different cell types. Because of their small size, cell-binding peptides such as these could be incorporated into biol. or phys. gene therapy vectors. In addn., peptide-presenting phage themselves may also be candidates for gene therapy vectors.

IT Peptides, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(cell-specific ligands; selection of cell-binding peptides from random peptide-presenting phage libraries for cell-targeting gene therapy vectors)

IT Animal cell
Fibroblast
Myoblast
(peptide ligands for; selection of cell-binding peptides from random peptide-presenting phage libraries for cell-targeting gene therapy vectors)

IT Virus, bacterial
(fd, display library vector; selection of cell-binding peptides from random peptide-presenting phage libraries for cell-targeting gene therapy vectors)

IT Proteins, specific or class
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(gene III, **fusion** products, phage display library; selection of cell-binding peptides from random peptide-presenting phage libraries for cell-targeting gene therapy vectors)

IT Therapeutics
(geno-, selection of cell-binding peptides from random peptide-presenting phage libraries for cell-targeting gene therapy vectors)

IT Muscle
(myotubule, peptide ligands for; selection of cell-binding peptides from random peptide-presenting phage libraries for cell-targeting gene therapy vectors)

IT 175479-86-4 175479-87-5 175479-88-6 175479-89-7 175479-90-0
175479-91-1 175479-92-2 175479-93-3 175479-94-4 175479-95-5
175479-96-6 175479-97-7 175479-98-8 175479-99-9 175480-00-9
175480-01-0 175480-02-1 175480-03-2 175480-04-3
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(fibroblast-specific peptide ligand; selection of cell-binding peptides from random peptide-presenting phage libraries for cell-targeting gene therapy vectors)

IT 175480-05-4 175480-06-5
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(myotube-specific peptide ligand; selection of cell-binding peptides from random peptide-presenting phage libraries for cell-targeting gene therapy vectors)

L10 ANSWER 10 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:149255 BIOSIS
DN PREV200300149255
TI Metabolically **biotinylated** gene therapy vectors for vector targeting and purification.
AU Parrott, M. Brandon (1); **Barry, Michael A.**
CS (1) Department of Immunology, Rice University, Houston, TX, USA USA
SO Cancer Gene Therapy, (January 2003, 2003) Vol. 10, No. Supplement 1, pp. S22-S23. print.

Meeting Info.: Eleventh International Conference on Gene Therapy of Cancer San Diego, CA, USA December 12-14, 2002
ISSN: 0929-1903.
DT Conference
LA English
IT Major Concepts
Bioprocess Engineering; Cell Biology; Medical Genetics (Allied Medical Sciences); Methods and Techniques; Molecular Genetics (Biochemistry and Molecular Biophysics); Oncology (Human Medicine, Medical Sciences); Pharmacology
IT Parts, Structures, & Systems of Organisms
tumor cell
IT Diseases
cancer: drug therapy, genetics, neoplastic disease
IT Chemicals & Biochemicals
Ad-Fiber-BAP [Ad-fiber-**biotin** acceptor peptide]; CD59; CD71; **biotin** acceptor peptide; **biotinylated** vector-combined targeting ligand
IT Alternate Indexing
Neoplasms (MeSH)
IT Methods & Equipment
gene transduction: genetic techniques, laboratory techniques; metabolically **biotinylated** vector gene therapy: clinical techniques, genetic techniques, therapeutic and prophylactic techniques; vector purification: applied and field techniques; viral gene therapy vector purification: applied and field techniques; viral gene therapy vector targeting: applied and field techniques
IT Miscellaneous Descriptors
Meeting Abstract
ORGN Super Taxa
Adenoviridae: dsDNA Viruses, Viruses, Microorganisms; Hominidae: Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
Adenovirus (Adenoviridae): Ad-Fiber-BAP, gene vector, wild-type; K562 cell line (Hominidae): erythroleukemia cell; human (Hominidae): patient
ORGN Organism Superterms
Animals; Chordates; Double-Stranded DNA Viruses; Humans; Mammals; Microorganisms; Primates; Vertebrates; Viruses

L10 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:607316 BIOSIS
DN PREV200200607316
TI Metabolically **biotinylated** gene therapy vectors.
AU **Barry, M. A.** (1); Parrott, M. B. (1)
CS (1) Molecular and Human Genetics, Immunology, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX USA
SO American Journal of Human Genetics, (October, 2002) Vol. 71, No. 4 Supplement, pp. 583. http://www.journals.uchicago.edu/AJHG/home.html. print.
Meeting Info.: 52nd Annual Meeting of the American Society of Human Genetics Baltimore, MD, USA October 15-19, 2002 American Society of Human Genetics
. ISSN: 0002-9297.
DT Conference
LA English
IT Major Concepts
Cell Biology; Molecular Genetics (Biochemistry and Molecular Biophysics)
IT Chemicals & Biochemicals
CD59; CD71; **biotin** acceptor peptide; holocarboxylase

synthetase
IT Methods & Equipment
flow cytometry: cell analytical method; gene therapy: molecular genetic method
IT Miscellaneous Descriptors
Meeting Abstract
ORGN Super Taxa
Hominidae: Primates, Mammalia, Vertebrata, Chordata, Animalia; Mammalia: Vertebrata, Chordata, Animalia; Viruses: Microorganisms
ORGN Organism Name
K562 cell line (Hominidae); mammal (Mammalia); virus (Viruses): metabolically **biotinylated** vector
ORGN Organism Superterms
Animals; Chordates; Humans; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Primates; Vertebrates; Viruses
RN 39419-81-3 (HOLOCARBOXYLASE SYNTHETASE)

L10 ANSWER 12 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:579044 BIOSIS
DN PREV200200579044
TI Targeting adenovectors to hemopoietic cells.
AU Yotnda, Patricia (1); Onishi, H. (1); Heslop, H. E. (1); Chen, D. (1); Chiu, Wah (1); Piedra, P. A. (1); Takahashi, S. (1); **Barry, M. (1)** ; Davis, A. (1); Templeton, N. Smyth (1); Brenner, M. K. (1)
CS (1) Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX USA
SO Blood Cells Molecules and Diseases, (May June, 2002) Vol. 28, No. 3, pp. 347. http://www.academicpress.com/bcmd. print.
Meeting Info.: Third Conference on Stem Cell Gene Therapy: Biology and Technology Rockville, MD, USA March 21-23, 2002
ISSN: 1079-9796.
DT Conference
LA English
IT Major Concepts
Blood and Lymphatics (Transport and Circulation); Immune System (Chemical Coordination and Homeostasis); Methods and Techniques; Molecular Genetics (Biochemistry and Molecular Biophysics)
IT Parts, Structures, & Systems of Organisms
hematopoietic progenitor cell: blood and lymphatics
IT Chemicals & Biochemicals
CD40L; immunomodulatory genes; interleukin-2
IT Methods & Equipment
adenoviral vector-mediated hematopoietic cell targeting: gene therapy method, gene transfer method, therapeutic method
IT Miscellaneous Descriptors
Meeting Abstract
ORGN Super Taxa
Adenoviridae: Animal Viruses, Viruses, Microorganisms
ORGN Organism Name
chimeric Ad5/F35 [adenovirus] (Adenoviridae): gene vector
ORGN Organism Superterms
Animal Viruses; Microorganisms; Viruses

L10 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:382284 BIOSIS
DN PREV199900382284
TI Recovery of functional response in the nucleus of the solitary tract after peripheral gustatory nerve crush and regeneration.
AU **Barry, Michael A. (1)**
CS (1) Dept. of BioStructure and Function, University of Connecticut Health

Center, Farmington, CT, 06030-3705 USA
SO Journal of Neurophysiology (Bethesda), (July, 1999) Vol. 82, No. 1, pp. 237-247.
ISSN: 0022-3077.
DT Article
LA English
SL English
AB Single-unit recording and transganglionic tracing techniques were used to assess the properties of, and inputs to, neurons within the rostral nucleus of the solitary tract (NST) after peripheral gustatory nerve injury and regeneration in adult hamsters (Mesocricetus auratus). Tastant-evoked responses were recorded from 43 neurons in animals in which the ipsilateral chorda tympani (CT) nerve was crushed 8 wk earlier (experimental animals) and from 46 neurons in unlesioned control animals. The 89 neurons were separated into three functional clusters named according to the best stimulus for neurons in the cluster: S, sucrose; N, sodium acetate; and H, HCl or KCl. Stimulus-evoked spike rates across all stimuli were 35.4 +- 4.4% lower in the experimental hamsters. The largest difference in evoked spike rates occurred for neurons in the H cluster, in which the response to KCl also was delayed relative to normal responses. The response of S-cluster units to sucrose and saccharin was also lower in the experimental animals. The mean response rate and the time course of response of neurons in the N cluster did not differ between the two groups. For each cluster, the spontaneous rates and mean response profiles across eight stimuli were very similar in the experimental and control animals, and the breadth of tuning hardly differed. In both groups, Na+ responses in the N cluster were amiloride sensitive, and responses to the water rinse after stimulation with HCl were common in the S cluster. At 8-20 wk after nerve crush, **biotinylated** dextran tracing of the CT nerve revealed that the regenerated CT fibers did not sprout outside the normal terminal zone in the NST, but the density of the central terminal fibers was 36.9 +- 6.35% lower than normal. After CT nerve crush and regeneration, the overall reduction in taste-evoked spike rates in NST neurons is likely a consequence of this change in terminal fibers; this in turn likely results from the known reduction in CT fibers regenerating past the crush site. In the face of this reduction, the normal taste-evoked spike rate in N-cluster neurons requires explanation. The observed recovery of normal specificity could be mediated by a restoration of specific connections by primary afferent fibers peripherally and centrally or by central compensatory mechanisms.
IT Major Concepts
Nervous System (Neural Coordination)
IT Parts, Structures, & Systems of Organisms
chorda tympani: nervous system; neurons: nervous system; nucleus of the solitary tract: functional response, nervous system; peripheral gustatory nerve: crush, recovery, nervous system
IT Miscellaneous Descriptors
behavior; nerve regeneration
ORGN Super Taxa
Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
Mesocricetus auratus [hamster] (Cricetidae)
ORGN Organism Superterms
Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates

L10 ANSWER 14 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1995:14855 BIOSIS
DN PREV199598029155
TI Central connectivity of the hamster gustatory nerves demonstrated with

biotinylated dextrans.
AU Knox, A. P.; **Barry, M. A.**
CS Univ. Conn. Health Cent., Farmington, CT 06030 USA
SO Chemical Senses, (1994) Vol. 19, No. 5, pp. 499.
Meeting Info.: Sixteenth Annual Meeting of the Association for Chemoreception Sciences Sarasota, Florida, USA April 1994
ISSN: 0379-864X.
DT Conference
LA English
IT Major Concepts
Dental and Oral System (Ingestion and Assimilation); Nervous System (Neural Coordination); Sense Organs (Sensory Reception)
IT Chemicals & Biochemicals
DEXTRANS
IT Miscellaneous Descriptors
CHORDA TYMPANI; GLOSSOPHARYNGEAL NERVE; MEETING ABSTRACT; SUPERIOR SALIVARY NUCLEUS; TASTE INFORMATION
ORGN Super Taxa
Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
Cricetidae (Cricetidae)
ORGN Organism Superterms
animals; chordates; mammals; nonhuman vertebrates; nonhuman mammals; rodents; vertebrates
RN 9004-54-0 (DEXTRANS)

L10 ANSWER 15 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1994:53756 BIOSIS
DN PREV199497066756
TI Brainstem connectivity of the hamster chorda tympani demonstrated with **biotinylated** dextrans.
AU Knox, A. P.; **Barry, M. A.**
CS Dep. BioStructure Function, Univ. Conn. Health Cent., Farmington, CT 06030-3705 USA
SO Society for Neuroscience Abstracts, (1993) Vol. 19, No. 1-3, pp. 1430.
Meeting Info.: 23rd Annual Meeting of the Society for Neuroscience Washington, D.C., USA November 7-12, 1993
ISSN: 0190-5295.
DT Conference
LA English
IT Major Concepts
Morphology; Nervous System (Neural Coordination)
IT Chemicals & Biochemicals
DEXTRANS
IT Miscellaneous Descriptors
FACIAL NERVE; MEETING ABSTRACT; MEETING POSTER
ORGN Super Taxa
Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
Cricetidae (Cricetidae)
ORGN Organism Superterms
animals; chordates; mammals; nonhuman vertebrates; nonhuman mammals; rodents; vertebrates
RN 9004-54-0 (DEXTRANS)

L10 ANSWER 16 OF 16 MEDLINE
AN 2000253236 MEDLINE
DN 20253236 PubMed ID: 10790424
TI M11L: a novel mitochondria-localized protein of myxoma virus that blocks apoptosis of infected leukocytes.

AU Everett H; **Barry M**; Lee S F; Sun X; Graham K; Stone J; Bleackley R C; McFadden G
CS Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.
SO JOURNAL OF EXPERIMENTAL MEDICINE, (2000 May 1) 191 (9) 1487-98.
Journal code: 2985109R. ISSN: 0022-1007.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200005
ED Entered STN: 20000613
Last Updated on STN: 20000613
Entered Medline: 20000530
AB M11L, a novel 166-amino acid membrane-associated protein expressed by the poxvirus, myxoma virus, was previously found to modulate apoptosis after infection of rabbit leukocytes. Furthermore, infection of rabbits with an M11L knockout virus unexpectedly produced lesions with a profound proinflammatory phenotype. We show here that M11L is antiapoptotic when expressed independently of other viral proteins, and is directed specifically to mitochondria by a short COOH-terminal region that is necessary and sufficient for targeting. This targeting region consists of a hydrophobic domain flanked by basic amino acid residues, adjacent to a positively charged tail. M11L blocks staurosporine-induced apoptosis by preventing mitochondria from undergoing a permeability transition, and the mitochondrial localization of this protein is essential for this function. We show that M11L is specifically required to inhibit the apoptotic response of monocytes/macrophages during virus infection, as cells of this lineage undergo apoptosis when infected with the M11L knockout virus. As monocyte apoptosis is uniquely proinflammatory, we propose that this observation reconciles the paradoxical proapoptotic and proinflammatory phenotypes of the M11L knockout virus. We suggest that apoptosis of tissue macrophages represents an important antiviral defense, and that the inhibition of apoptosis by viral proteins can be directed in a cell-specific fashion.

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=> d his

     (FILE 'HCAPLUS' ENTERED AT 13:01:21 ON 01 MAY 2003)
                DEL HIS Y
L1           887 S (P OR PROPIONIBACTER?) (2W) SHERMANII
L2          2569 S PROPIONIBACTER?
L3           124 S TRANSCARBOXYLAS?
L4            49 S L1 AND L3
L5            10 S L4 AND (FUSION OR CHIMER? OR RECOMBIN?)

     FILE 'WPIDS' ENTERED AT 13:03:16 ON 01 MAY 2003

     FILE 'HCAPLUS' ENTERED AT 13:04:04 ON 01 MAY 2003
L6             0 S PSTCD OR PSTCD/AB

     FILE 'WPIDS' ENTERED AT 13:04:48 ON 01 MAY 2003
L7            64 S (P OR PROPIONIBACTER?) (2W) SHERMANII
L8             3 S L7 AND (FUSION OR CHIMER? OR RECOMBIN?)
L9             3 S L7 AND TRANSCARBOXYLA?
L10            3 S L8 OR L9

     FILE 'BIOSIS' ENTERED AT 13:06:00 ON 01 MAY 2003
L11          679 S (P OR PROPIONIBACTER?) (2W) SHERMANII
L12           52 S L11 AND TRANSCARBOXYLASE?
L13            6 S L12 AND (FUSION OR CHIMER? OR RECOMBIN?)
L14            0 S PSTCD

     FILE 'WPIDS' ENTERED AT 13:06:47 ON 01 MAY 2003
L15            1 S PSTCD
L16            3 S L15 OR L10

     FILE 'WPIDS, BIOSIS, HCAPLUS' ENTERED AT 13:07:15 ON 01 MAY 2003
L17           16 DUP REM L16 L13 L5 (3 DUPLICATES REMOVED)

=> d que l17
L1           887 SEA FILE=HCAPLUS ABB=ON  PLU=ON  (P/OBI OR PROPIONIBACTER?/OBI)
                   (2W) SHERMANII/OBI
L3           124 SEA FILE=HCAPLUS ABB=ON  PLU=ON  TRANSCARBOXYLAS?/OBI
L4            49 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 AND L3
L5            10 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L4 AND (FUSION/OBI OR
                   CHIMER?/OBI OR RECOMBIN?/OBI)
L7            64 SEA FILE=WPIDS ABB=ON  PLU=ON  (P OR PROPIONIBACTER?) (2W)
                   SHERMANII
L8             3 SEA FILE=WPIDS ABB=ON  PLU=ON  L7 AND (FUSION OR CHIMER? OR
                   RECOMBIN?)
L9             3 SEA FILE=WPIDS ABB=ON  PLU=ON  L7 AND TRANSCARBOXYLA?
L10            3 SEA FILE=WPIDS ABB=ON  PLU=ON  L8 OR L9
L11          679 SEA FILE=BIOSIS ABB=ON  PLU=ON  (P OR PROPIONIBACTER?) (2W)
                   SHERMANII
L12           52 SEA FILE=BIOSIS ABB=ON  PLU=ON  L11 AND TRANSCARBOXYLASE?
L13            6 SEA FILE=BIOSIS ABB=ON  PLU=ON  L12 AND (FUSION OR CHIMER? OR
                   RECOMBIN?)
L15            1 SEA FILE=WPIDS ABB=ON  PLU=ON  PSTCD
L16            3 SEA FILE=WPIDS ABB=ON  PLU=ON  L15 OR L10
L17           16 DUP REM L16 L13 L5 (3 DUPLICATES REMOVED)


=> d bib ab it 1-16

L17  ANSWER 1 OF 16  WPIDS (C) 2003 THOMSON DERWENT
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AN 2003-165810 [16] WPIDS
DNN N2003-130921 DNC C2003-042975
TI Novel **fusion** protein useful for targeting desired protein to cell in culture or in the body of subject, comprises biotinylation-competent protein/peptide, or biotin acceptor peptide (BAP), and desired polypeptide.
DC B04 D16 S03
IN BARRY, M A; PARROTT, M B
PA (BAYU) BAYLOR COLLEGE MEDICINE
CYC 1
PI US 2002142355 A1 20021003 (200316)* 13p
ADT US 2002142355 A1 Provisional US 2000-247965P 20001114, US 2001-987485 20011114
PRAI US 2000-247965P 20001114; US 2001-987485 20011114
AB US2002142355 A UPAB: 20030307
NOVELTY - A **fusion** protein (I) consisting essentially of a biotinylation-competent protein or peptide, or a biotin acceptor peptide (BAP), and a polypeptide of interest, where the biotinylation-competent protein or peptide, or BAP is joined directly to the N- or C-terminal end of the polypeptide of interest, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a polynucleotide vector (II) for expressing protein comprising a coding region consisting of nucleotides encoding (I), and a promoter active in mammalian cells and operably linked to the coding region; and

(2) biotin-labeling (M) a virus, involves replicating the virus in a mammalian host cell, where the host cell expresses a biotin ligase and has been engineered to express (II).

USE - (I) is useful for targeting a protein of interest which is on the surface of a virus, to a cell in culture in the body of a subject. The method involves binding avidin to the surface of the cell, biotinylating (I), where the protein of interest is joined to the biotinylation-competent protein or peptide, and administering the biotinylated protein to either the medium surrounding the cell in culture or to the subject. The avidin is bound to the surface of the cell by attaching avidin to a ligand that binds to a receptor located on the surface of the cell, and administering the avidin/ligand molecule either to the medium surrounding the cell in culture or to the subject. The protein of interest is used to target the virus to the cell. (II) is useful for biotinylating a polypeptide of interest secreted by a mammalian host cell, by expressing (II) in a mammalian host cell in vivo or in vitro. The cell is a Chinese hamster ovary (CHO) cell in culture, and is engineered to express a distinct **fusion** protein consisting of a biotin ligase (e.g. BirA) directly linked to a leader sequence (e.g. Igkappa secretory leader) that promotes secretion from the host cell (all claimed).

(I) is useful for drug and gene therapy targeting. The biotin labeled proteins are useful for delivering nucleic acids to cell in vivo. (M) is useful for rapidly purifying virus, for attaching other compounds to the virus, for modifying the virus's ability to transduce cells in vivo and ex vivo, and for directing the virus to specific avidin-tagged sites in a patient's body.

ADVANTAGE - (II) effectively labels polypeptides with biotin.
Dwg.0/1

L17 ANSWER 2 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 2002:199169 HCAPLUS
DN 137:75477
TI Metabolic biotinylation of **recombinant** proteins in mammalian cells and in mice
AU Parrott, M. Brandon; Barry, Michael A.

CS Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX, 77030, USA
SO Molecular Therapy (2000), 1(1), 96-104
CODEN: MTOHCK; ISSN: 1525-0016
PB Academic Press
DT Journal
LA English
AB The avidin-biotin system is a fundamental technol. in biomedicine for immunolocalization, imaging, nucleic acid blotting, and protein labeling. While this technol. is robust, it is limited by the fact that mammalian proteins must be expressed and purified prior to chem. biotinylation using crosslinking agents which modify proteins at random locations to heterogeneous levels and can inactivate protein function. To circumvent this limitation, we demonstrate the ability to metabolically biotinylate tagged proteins in mammalian cells and in mice using the endogenous biotinylation enzymes of the host. Endogenously biotinylated proteins were readily purified from mammalian cells using monomeric avidin and eluted under nondenaturing conditions using only biotin as the releasing agent. This technol. should allow recombinant proteins and fragile protein complexes to be produced and purified from mammalian cells as well as from transgenic plants and animals. In addn., this technol. may be particularly useful for cell-targeting applications in which proteins or viral gene therapy vectors can be biotinylated at genetically defined sites for combination with other targeting moieties complexed with avidin.

IT Post-translational processing
(biotinylation; metabolic biotinylation and purifn. of **recombinant** proteins fused to **P. shermanii transcarboxylase** domain in mammalian cells and mice)
IT Cricetulus griseus
Mouse
Propionibacterium shermanii
Protein motifs
(metabolic biotinylation and purifn. of **recombinant** proteins fused to **P. shermanii transcarboxylase** domain in mammalian cells and mice)
IT **Fusion** proteins (**chimeric** proteins)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(metabolic biotinylation and purifn. of **recombinant** proteins fused to **P. shermanii transcarboxylase** domain in mammalian cells and mice)
IT Protein sequences
(of **transcarboxylase**; metabolic biotinylation and purifn. of **recombinant** proteins fused to **P. shermanii transcarboxylase** domain in mammalian cells and mice)
IT 58-85-5, Biotin
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(metabolic biotinylation and purifn. of **recombinant** proteins fused to **P. shermanii transcarboxylase** domain in mammalian cells and mice)
IT 9029-86-1, **Transcarboxylase**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(metabolic biotinylation and purifn. of **recombinant** proteins fused to **P. shermanii transcarboxylase** domain in mammalian cells and mice)
RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 3 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 1999:608309 HCAPLUS
DN 132:1507
TI Expression and Biotinylation of a Mutant of the **Transcarboxylase** Carrier Protein from Propioni shermanii
AU Jank, Matthias M.; Bokorny, Stefan; Rohm, Klaus - Heinrich; Berger, Stefan
CS Institut fur Analytische Chemie, Universitat Leipzig, Leipzig, 04103, Germany
SO Protein Expression and Purification (1999), 17(1), 123-127
CODEN: PEXPEJ; ISSN: 1046-5928
PB Academic Press
DT Journal
LA English
AB A deletion mutant (residues 10 to 48 cut) of the biotinyl subunit (tcc) from the enzyme transcarboxylase (EC 2.1.3.1) of Propioni shermanii was over-expressed in Escherichia coli. Complete biotinylation of the protein was achieved by addn. of exogenous biotin and co-expression of the biotin holoenzyme synthetase (EC 6.3.4.15.) from E. coli. The transcription of both genes was put under control of different operators/promoters, thus achieving independent control of expression levels and optimized yields of the holo-tcc. Bacteria were grown in a biotin-supplemented minimal medium (M9) that contained [13C]glucose as the carbon source and [15N]NH4Cl as the sole nitrogen source. The target protein could be purified to homogeneity by ion-exchange chromatog. and concd. to NMR-suitable concns. (2 mM) without aggregation. (c) 1999 Academic Press.
IT Biotinylation
Propionibacterium shermanii
(expression and biotinylation of a mutant of the **transcarboxylase** carrier protein from Propioni shermanii)
IT 9029-86-1P, **Transcarboxylase**
RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); PROC (Process)
(**recombinant**; expression and biotinylation of a mutant of the **transcarboxylase** carrier protein from Propioni shermanii)
RE.CNT 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 4 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 1998:640407 HCAPLUS
DN 129:272665
TI High throughput assays using **fusion** proteins for screening binding compounds and protease inhibitors
IN Hermes, Jeffrey D.; Salowe, Scott P.; Sinclair, Peter J.
PA Merck & Co., Inc., USA
SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9841856	A1	19980924	WO 1998-US4610	19980310

W: CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
PRAI US 1997-40795P P 19970314
AB This application describes a high throughput assay for screening compds. which are capable of binding to a fusion protein which consists of a target protein and an FK506-binding protein. This application also describes an assay for screening compds. which inhibit a protease. A

FK506-binding protein-ZAP70 tandem SH2 domains fusion protein was recombinantly prepd., expressed in Escherichia coli, and purified by affinity chromatog. on agarose-immobilized avidin having bound biotinylated phosphopeptide derived from the .zeta.1 ITAM sequence of the human T-cell receptor. Inhibitors of the fusion protein are screened using the biotinylphosphopeptide, the fusion protein, and europium cryptate-labeled FK506 analog in wells of a 96-well black microplate. The fluorescence ratio is measured in a Packard Discovery homogeneous time-resolved fluorescence analyzer.

IT Proteins, specific or class
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(FKBP (FK 506-binding protein), **fusion** protein contg.; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT TCR (T cell receptors)
RL: MSC (Miscellaneous)
(ITAM (immunoreceptor tyrosine-based activation motif) sequence of, biotinylated phosphopeptide from, in **fusion** protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Protein motifs
(ITAM (immunoreceptor tyrosine-based activation motif), biotinylated phosphopeptide from, in **fusion** protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Protein motifs
(PH domain, **fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Protein motifs
(SH1 domain, **fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Protein motifs
(SH2 domain, **fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Protein motifs
(SH3 domain, **fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Phosphoproteins
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(ZAP-70 (TCR receptor .zeta.-chain-assocd., 70,000-mol.-wt.), SH2 domain, **fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Avidins
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)
(agarose-immobilized, complexes with biotinylated ITAM phosphopeptide, as affinity matrix for purifn. of **fusion** proteins; high throughput assays using **fusion** proteins for screening binding

compds. and protease inhibitors)

IT Escherichia coli
(biotin carboxy carrier protein of acetyl-CoA carboxylase of, as reporter protein in **fusion** protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT **Propionibacterium shermanii**
(biotin-carrier subunit of **transcarboxylase** of, as reporter protein in **fusion** protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Ligands
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(biotinylated or acceptor-labeled; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Phosphopeptides
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(biotinylated, from ITAM sequence, in **fusion** protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors).

IT Proteins, general, biological studies
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); RCT (Reactant); ANST (Analytical study); BIOL (Biological study); PROC (Process); RACT (Reactant or reagent); USES (Uses)
(biotinylated, **fusion** protein contg., as reporter protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Tumor necrosis factors
RL: BPR (Biological process); BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); PROC (Process); RACT (Reactant or reagent)
(converting enzyme for, inhibitors; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Rare earth metals, biological studies
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(coupled with cryptate-contg. mol., as donor-labeled ligands; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Signal transduction, biological
(**fusion** protein contg. protein domains of, as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Nuclear receptors
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(**fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Genetic vectors
Molecular cloning

Protein sequences
(high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT **Fusion** proteins (**chimeric** proteins)
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); PUR (Purification or recovery); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Peptides, biological studies
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); RCT (Reactant); ANST (Analytical study); BIOL (Biological study); PROC (Process); RACT (Reactant or reagent); USES (Uses)
(linker, **fusion** protein contg.; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Phosphoproteins
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(p72syk, SH2 domain, **fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Protein motifs
(signal transduction domains, **fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Proteins, general, biological studies
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); RCT (Reactant); ANST (Analytical study); BIOL (Biological study); PROC (Process); RACT (Reactant or reagent); USES (Uses)
(target or reporter, **fusion** protein contg.; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT Fluorometry
(time-resolved; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 125433-96-7
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(FK506 analog labeled with, as donor-labeled ligands; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 209212-23-7 209212-27-1
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(PCR primer for FK506-binding protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 209212-43-1 213987-27-0
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(PCR primer for SH2 domain of Lck; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 209212-36-2 209212-39-5
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(PCR primer for SH2 domain of Syk; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 209212-30-6 209212-34-0
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(PCR primer for SH2 domain of ZAP70; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 114051-78-4P, Lck tyrosine kinase
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(SH2 domain, **fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 188796-99-8
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(amino acid sequence, of human T-cell receptor Zeta 1, **fusion** protein contg.; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 9012-36-6D, Agarose, avidin conjugates, complexes with biotinylated ITAM phosphopeptide 213611-43-9D, complexes with agarose-immobilized avidin 213611-44-0D, complexes with agarose-immobilized avidin
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)
(as affinity matrix for purifn. of **fusion** proteins; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 53123-88-9D, Rapamycin, analogs, labeled with europium cryptate 104987-11-3D, FK506, analogs, labeled with europium cryptate
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(as donor-labeled ligands; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 9023-93-2, Acetyl-CoA carboxylase
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); RCT (Reactant); ANST (Analytical study); BIOL (Biological study); PROC (Process); RACT (Reactant or reagent); USES (Uses)
(biotin carboxy carrier protein of, of Escherichia coli, as reporter protein in **fusion** protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)

IT 37340-30-0, **Transcarboxylase**
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); RCT (Reactant); ANST (Analytical study); BIOL (Biological study); PROC (Process); RACT (Reactant or reagent); USES (Uses)
(biotin-carrier subunit of, of Propionibacterium shermannii, as reporter protein in **fusion** protein; high throughput assays using **fusion** proteins for screening binding compds. and

protease inhibitors)
IT 104987-11-3, FK506
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**fusion** protein contg. protein binding to; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)
IT 79747-53-8, Tyrosine phosphatase
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(**fusion** protein contg., as target protein; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)
IT 58-85-5D, Biotin, conjugates 9013-20-1D, Streptavidin, acceptor-labeled 213833-07-9, SA-XL 665
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)
IT 9001-92-7, Protease 9002-04-4, Thrombin 151769-16-3, TNF.alpha.-converting enzyme
RL: BPR (Biological process); BSU (Biological study, unclassified); CAT (Catalyst use); BIOL (Biological study); PROC (Process); USES (Uses)
(inhibitors; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)
IT 144114-21-6, Retropepsin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(inhibitors; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)
IT 146669-16-1
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(mol. contg., coupled to lanthanide, as donor-labeled ligands; high throughput assays using **fusion** proteins for screening binding compds. and protease inhibitors)
RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 5 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:338336 BIOSIS
DN PREV199699060692
TI [redacted] as a sensitive indicator of protein secretion and membrane protein insertion.
AU Jander, Georg; Cronan., John E., Jr.; Beckwith, Jon (1)
CS [redacted]
SO [redacted] Vol. 178, No. 11, pp. 3049-3058. ISSN: 0021-9193.
DT Article
LA English
AB Escherichia coli biotin ligase is a cytoplasmic protein which specifically biotinylates the biotin-accepting domains from a variety of organisms. This in vivo biotinylation can be used as a sensitive signal to study protein secretion and membrane protein insertion. When the biotin-accepting domain from the 1.3S subunit of **Propionibacterium shermanii transcarboxylase** (PSBT) is translationally fused to the periplasmic proteins alkaline phosphatase and maltose-binding protein, there is little or no biotinylation of PSBT in wild-type E. coli.

Inhibition of SecA with sodium azide and mutations in SecB, SecD, and SecF, all of which slow down protein secretion, result in biotinylation of PSBT. When PSBT is fused to the E. coli inner membrane protein MalF, it acts as a topological marker: **fusions** to cytoplasmic domains of MalF are biotinylated, and **fusions** to periplasmic domains are generally not biotinylated. If SecA is inhibited by sodium azide or if the SecE in the cell is depleted, then the insertion of the MalF second periplasmic domain is slowed down enough that PSBT **fusions** in this part of the protein become biotinylated. Compared with other protein **fusions** that have been used to study protein translocation, PSBT **fusions** have the advantage that they can be used to study the rate of the insertion process.

IT Major Concepts
Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics); Genetics; Membranes (Cell Biology); Metabolism; Physiology

IT Chemicals & Biochemicals
TRANSCARBOXYLASE; ALKALINE PHOSPHATASE

IT Miscellaneous Descriptors
ALKALINE PHOSPHATASE; BIOTIN LIGASE; BIOTINYLATION; CELL BIOLOGY/MEMBRANES; GENETIC METHOD; MALF INNER MEMBRANE PROTEIN; MALTOSE-BINDING PROTEIN; MEMBRANE INSERTION; METABOLISM; MUTATIONAL ANALYSIS; PROTEIN; SECRETION; **TRANSCARBOXYLASE**

ORGN Super Taxa
Enterobacteriaceae: Eubacteria, Bacteria; Irregular Nonsporing Gram-Positive Rods: Eubacteria, Bacteria

ORGN Organism Name
irregular nonsporing gram-positive rods (Irregular Nonsporing Gram-Positive Rods); Escherichia coli (Enterobacteriaceae); **Propionibacterium shermanii** (Irregular Nonsporing Gram-Positive Rods)

ORGN Organism Superterms
bacteria; eubacteria; microorganisms

RN 9029-86-1Q (**TRANSCARBOXYLASE**)
9067-78-1Q (**TRANSCARBOXYLASE**)
37340-30-0Q (**TRANSCARBOXYLASE**)
9001-78-9 (ALKALINE PHOSPHATASE)

L17 ANSWER 6 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1995:176829 BIOSIS
DN PREV199598191129
TI Functional activity of biotinylated human neurokinin 1 receptor **fusion** expressed in the Semliki Forest virus system.
AU Lundstrom, Kenneth (1); Vargas, Alfredo; Allet, Bernard
CS (1) Glaxo Inst. Mol. Biol., 14 Chemin des Aulx, 1228 Plan-les-Ouates, Geneva Switzerland
SO Biochemical and Biophysical Research Communications, (1995) Vol. 208, No. 1, pp. 260-266.
ISSN: 0006-291X.
DT Article
LA English
AB The 1.3 S biotinylatable subunit of Proprionibacterium shermanii **transcarboxylase** complex was fused to the C-terminus of the human neurokinin 1 receptor gene and introduced into the Semliki Forest virus expression vector pSFV1. RNA transcribed from pSFV1-NK1-blot and pSFV-Helper2 was coelectroporated into BHK cells permitting in vivo packaging of **recombinant** virus. Infection of BHK and CHO cells with SFV-NK1-biot virus yielded high level of the **fusion** receptor as detected by metabolic labeling, immunoblotting with streptavidin alkaline phosphatase and binding to substance P. Like native

receptor, the biotinylated receptor **fusion** was able to stimulate Ca-2+ mobilization in infected CHO cells, indicating functional coupling to guanine-nucleotide-binding proteins.

IT Major Concepts
Endocrine System (Chemical Coordination and Homeostasis); Genetics; Membranes (Cell Biology); Nervous System (Neural Coordination)

IT Chemicals & Biochemicals
SUBSTANCE P

IT Miscellaneous Descriptors
G-PROTEIN; GENETIC ENGINEERING; SUBSTANCE P

ORGN Super Taxa
Animal Viruses - General: Viruses; Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Hominidae: Primates, Mammalia, Vertebrata, Chordata, Animalia; Irregular Nonsporing Gram-Positive Rods: Eubacteria, Bacteria; Togaviridae: Viruses

ORGN Organism Name
arbovirus (Animal Viruses - General); hamster (Cricetidae); irregular nonsporing gram-positive rods (Irregular Nonsporing Gram-Positive Rods); Hominidae (Hominidae); **Propionibacterium shermanii** (Irregular Nonsporing Gram-Positive Rods); Togaviridae (Togaviridae)

ORGN Organism Superterms
animals; bacteria; chordates; eubacteria; humans; mammals; microorganisms; nonhuman mammals; nonhuman vertebrates; primates; rodents; vertebrates; viruses

RN 33507-63-0 (SUBSTANCE P)

L17 ANSWER 7 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 1994:390755 BIOSIS

DN PREV199497403755

TI Mutations participating in interallelic complementation in propionic acidemia.

AU Gravel, R. A. (1); Akerman, B. R.; Lamhonwah, A.-M.; Loyer, M.; Leon-Del-Rio, A.; Italiano, I.

CS (1) McGill Univ.-Montreal Children's Hosp. Res. Inst., 2300 Tupper St., Montreal, PQ H3H 1P3 Canada

SO American Journal of Human Genetics, (1994) Vol. 55, No. 1, pp. 51-58. ISSN: 0002-9297.

DT Article

LA English

AB Deficiency of propionyl-CoA carboxylase (PCC; alpha-4-beta-4) results in the rare, autosomal recessive disease propionic acidemia. Cell **fusion** experiments have revealed two complementation groups, pccA and pccB, corresponding to defects of the PCCA (alpha-subunit) and PCCB (beta-subunit) genes, respectively. The pccBCC group includes subgroups, pccB and pccC, which are thought to reflect interallelic complementation between certain mutations of the PCCB gene. In this study, we have identified the mutations in two pccB, one pccC, and two pccBC cell lines and have deduced those alleles participating in interallelic complementation. One pccB line was a compound heterozygote of Pro228Leu and Asn536Asp. The latter mutation was also detected in a noncomplementing pccBC line. This leaves Pro228Leu responsible for complementation in the pccB cells. The second pccB line contained an insertional duplication, dupKICK140-143, and a splice mutation IVS + 1 G fwdarw T, located after Lys466. We suggest that the dupKICK mutation is the complementing allele, since the second allele is incompatible with normal splicing. The pccC line studied was homozygous for Arg410Trp, which is necessarily the complementing allele in that line. For a second pccC line, we previously had proposed that DELTA-Ile408 was the complementing allele. We now show that its second allele, "Ins cntdot Del," a 14-bp deletion replaced by a

12-bp insertion beginning at codon 407, fails to complement in homozygous form. We conclude that the interallelic complementation results from mutations in domains that can interact between beta-subunits in the PCC heteromer to restore enzymatic function. On the basis of sequence homology with the **Propionibacterium shermanii transcarboxylase** 12S subunit, we suggest that the pccC domain, defined by Ile408 and Arg410, may involve the propionyl-CoA binding site.

IT Major Concepts
Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics); Genetics; Metabolism; Physiology

IT Chemicals & Biochemicals
PROPIONYL-COA CARBOXYLASE

IT Miscellaneous Descriptors
BACTERIAL ENZYME; INSERTIONAL DUPLICATION; PROPIONYL-COA CARBOXYLASE; SPLICE MUTATION

ORGN Super Taxa
Hominidae: Primates, Mammalia, Vertebrata, Chordata, Animalia; Irregular Nonsporing Gram-Positive Rods: Eubacteria, Bacteria

ORGN Organism Name
human (Hominidae); irregular nonsporing gram-positive rods (Irregular Nonsporing Gram-Positive Rods); **Propionibacterium shermanii** (Irregular Nonsporing Gram-Positive Rods)

ORGN Organism Superterms
animals; bacteria; chordates; eubacteria; humans; mammals; microorganisms; primates; vertebrates

RN 9023-94-3Q (PROPIONYL-COA CARBOXYLASE)
37289-44-4Q (PROPIONYL-COA CARBOXYLASE)

L17 ANSWER 8 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 1993-336075 [42] WPIDS
CR 1990-375998 [50]
DNC C1993-148653
TI **Fusion** proteins having site for post-translation modification - utilised esp. for biotin, used to identify or isolate **fusion** proteins from mixts..
DC B04 D16
IN CRONAN, J E
PA (BIOT-N) BIOTECHNOLOGY RES & DEV CORP; (UNII) UNIV ILLINOIS FOUND
CYC 1
PI US 5252466 A 19931012 (199342)* 57p
ADT US 5252466 A CIP of US 1989-354266 19890519, US 1990-525568 19900518
PRAI US 1989-354266 19890519; US 1990-525568 19900518
AB US 5252466 A UPAB: 19940613
A transformed host cell into which DNA has been introduced, or progeny of the transformed host cell are claimed, the introduced DNA comprising (a) DNA coding for a **fusion** protein comprising (i) a first DNA sequence which codes for a protein or polypeptide having an amino acid sequence that allows for post-translation biotination of the **fusion** protein and (ii) a second DNA sequence joined end to end with first DNA sequence and in the same reading frame, the second DNA sequence encoding a selected protein or polypeptide, and (b) DNA coding for biotin ligase, the DNA coding for the **fusion** protein and the DNA coding for biotin ligase being operatively linked to expression control sequences.

The first DNA sequence may code for e.g. the 1.3 S subunit of **Propionibacterium shermanii transcarboxylase**, tomato biotin protein, the alpha subunit of K. pneumoniae oxalacetate decarboxylase, E.coli biotin carboxyl carrier protein or fragments of these proteins that allow for post-translation biotination of the **fusion** proteins.

USE/ADVANTAGE - The post-translation biotination provides a marker for the **fusion** protein that can be used, directly or indirectly, to identify the **fusion** protein or to isolate it from a mixt. of other materials such as host cell culture medium
Dwg.0/34

L17 ANSWER 9 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 1993:590528 BIOSIS
DN PREV199497009898
TI Identification and characterization of a factor which is essential for assembly of **transcarboxylase**.
AU Shenoy, Bhami C.; Xie, Yun; Sha, Dan; Samols, David (1)
CS (1) Dep. Biochem., Sch. Med., Case Western Reserve Univ., 10900 Euclid Ave., Cleveland, OH 44106-4935 USA
SO Biochemistry, (1993) Vol. 32, No. 40, pp. 10750-10756. ISSN: 0006-2960.
DT Article
LA English
AB **Transcarboxylase** (TC) from **Propionibacterium shermanii** is a biotin-containing enzyme which catalyzes the reversible transfer of a carboxyl group from methylmalonyl-CoA to pyruvate. It is composed of a central, hexameric 12S subunit with six outer, dimeric 5S subunits held in a stable 26S complex by twelve 1.3S biotinyl subunits. Each of these subunits has been cloned from the **P. shermanii** genome and expressed in Escherichia coli. The purified, expressed **recombinant** proteins are all indistinguishable from their authentic counterparts except for the **recombinant** 5S subunit (termed 5S WT), which does not form TC complexes or catalyze the overall **transcarboxylase** reaction. Circular dichroism and isoelectric focusing suggested differences existed between the authentic and E. coli-expressed 5S proteins. HPLC gel filtration was used to separate the authentic 5S dimer from additional components in the preparation. 5S dimer thus purified was unable to form TC complexes or catalyze the overall reaction, behaving identically to the **recombinant** 5S WT subunit. Fractions from the HPLC gel-filtration purification of authentic 5S were then added to 5S WT or 5S dimer, and one fraction was identified which catalyzed the assembly of TC complexes with either 5S preparation. This assembly activity was shown to be dependent on the concentration of this HPLC fraction. Assembly-promoting factor (APF) is heat-stable and probably a protein, on the basis of its protease susceptibility. Studies with APF and the other TC subunits demonstrate its ability to promote complex formation with 12S and 1.3S subunits. Since the APF was purified from crystals of 26S TC, we believe it to be a novel, previously unidentified subunit of **transcarboxylase**.
IT Major Concepts
Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics); Genetics; Metabolism; Methods and Techniques; Molecular Genetics (Biochemistry and Molecular Biophysics); Physiology
IT Chemicals & Biochemicals
TRANSCARBOXYLASE
IT Miscellaneous Descriptors
ANALYTICAL METHOD; ENZYME ACTIVITY; ENZYME SUBUNITS; EXPRESSION; GENETIC ENGINEERING; HIGH PERFORMANCE LIQUID CHROMATOGRAPHY; PURIFICATION METHOD; **RECOMBINANT** PROTEINS
ORGN Super Taxa
Enterobacteriaceae: Eubacteria, Bacteria; Irregular Nonsporing Gram-Positive Rods: Eubacteria, Bacteria
ORGN Organism Name
irregular nonsporing gram-positive rods (Irregular Nonsporing

Gram-Positive Rods); Escherichia coli (Enterobacteriaceae); **Propionibacterium shermanii** (Irregular Nonsporing Gram-Positive Rods)

ORGN Organism Superterms
bacteria; eubacteria; microorganisms

RN 9029-86-1Q (**TRANSCARBOXYLASE**)
9067-78-1Q (**TRANSCARBOXYLASE**)
37340-30-0Q (**TRANSCARBOXYLASE**)

L17 ANSWER 10 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 1994:3471 HCAPLUS
DN 120:3471
TI Primary structure of the monomer of the 12S subunit of **transcarboxylase** as deduced from DNA and characterization of the product expressed in Escherichia coli
AU Thornton, Charles G.; Kumar, Ganesh K.; Haase, F. Carl; Phillips, Nelson F. B.; Woo, Sang B.; Park, Vicki M.; Magner, William J.; Shenoy, Bhami C.; Wood, Harland G.; Samols, David
CS Dep. Biochem., Case West. Reserve Univ., Cleveland, OH, 44106-4935, USA
SO Journal of Bacteriology (1993), 175(17), 5301-8
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB Transcarboxylase from Propionibacterium shermanii is a complex biotin-contg. enzyme composed of 30 polypeptides of three different types: a hexameric central 12S subunit to which 6 outer 5S subunits are attached through 12 1.3S biotinyl subunits. The enzyme catalyzes a two-step reaction in which methylmalonyl CoA and pyruvate serve as substrates to form propionyl CoA (propionyl-CoA) and oxalacetate, the 12S subunit specifically catalyzing one of the two reactions. The authors report here the cloning, sequencing, and expression of the 12S subunit. The gene was identified by matching amino acid sequences derived from isolated authentic 12S peptides with the deduced sequence of an open reading frame present in a cloned P. shermanii genomic fragment known to contain the gene encoding the 1.3S biotinyl subunit. The cloned 12S gene encodes a protein of 604 amino acids and of Mr 65,545. The deduced sequence shows regions of extensive homol. with the .beta. subunit of mammalian propionyl-CoA carboxylase as well as regions of homol. with acetyl-CoA carboxylase from several species. Two genomic fragments were subcloned into pUC19 in an orientation such that the 12S open reading frame could be expressed from the lac promoter of the vector. Crude exts. prepd. from these cells contained an immunoreactive band on Western blots (immunoblots) which comigrated with authentic 12S. The Escherichia coli-expressed 12S was purified to apparent homogeneity by a three-step procedure and compared with authentic 12S from P. shermanii. Their quaternary structures were identical by electron microscopy, and the E. coli 12S prepn. was fully active in the reactions catalyzed by this subunit. It was concluded that the authors have cloned, sequenced, and expressed the 12S subunit which exists in a hexameric active form in E. coli.
IT Gene, microbial
RL: BIOL (Biological study)
(for **transcarboxylase** 12S subunit, of **Propionibacterium shermanii**, nucleotide sequence of)
IT Deoxyribonucleic acid sequences
(of **transcarboxylase** 12S subunit gene, of **Propionibacterium shermanii**)
IT Quaternary structure
(of **transcarboxylase** 12S subunit **recombinant** form, of **Propionibacterium shermanii**)

IT Protein sequences
(of **transcarboxylase** 12S subunit, of **Propionibacterium shermanii**)
IT 151689-38-2, **Transcarboxylase** (**Propionibacterium shermanii** clone pLac12S+1.35 precursor subunit 12S)(enzyme E.C. 2.1.3.1)
RL: BIOL (Biological study)
(amino acid sequence and functional expression in Escherichia coli of)
IT 144623-30-3, GenBank L04196
RL: PRP (Properties); BIOL (Biological study)
(nucleotide sequence of)

L17 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 1993:590490 BIOSIS
DN PREV199497009860
TI Purification and characterization of the **recombinant** 5 S subunit of **transcarboxylase** from Escherichia coli.
AU Xie, Yun; Shenoy, Bhami C.; Magner, William J.; Hejlik, Daniel P.; Samols, David (1)
CS (1) Dep. Biochem., Case Western Reserve Univ., Cleveland, OH 44106-4935 USA
SO Protein Expression and Purification, (1993) Vol. 4, No. 5, pp. 456-464.
ISSN: 1046-5928.
DT Article
LA English
AB **Transcarboxylase** from **Propionibacterium shermanii** is a biotin-containing enzyme which catalyzes the reversible transfer of a carboxyl group from methylmalonyl-CoA to pyruvate. It is composed of a central, hexameric 12 S subunit, 6 outer dimeric 5 S subunits which are held in a complex by 12 1.3 S biotinyl subunits. The **transcarboxylase** reaction requires two partial reactions, one of which is specific to 5 S. The cloning and expression of each of these subunits in Escherichia coli have been reported. We have designed a method for the purification of the 5 S subunit from an E. coli expression system. Protein purified to homogeneity by this method was shown to be active in the 5 S partial reaction, but unable to catalyze the overall **transcarboxylase** reaction. This protein was characterized as to its ability to form stable dimers, associate with the 1.3 S subunit in stable complexes referred to as 6 S, and assemble whole TC. The latter activity was shown to be lacking. The purified protein has a native molecular weight of 120 kDa and a subunit molecular weight of 60 kDa, consistent with the 5 S dimer. Plasma emission analysis of the metal content of the **recombinant** protein demonstrated the presence of both Co and Zn, comparable to the authentic protein. Fluorescence analysis verified the ability of the purified protein to bind substrates and 1.3 S subunits appropriately. Sequencing of the amino terminus and determination of the amino acid composition of the **recombinant** protein relative to that of the authentic subunit further verified the identity of the purified protein. In sum, the **recombinant** 5 S subunit was indistinguishable from authentic 5 S by all criteria except ability to form TC complexes and catalyze the overall reaction.
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Physiology
IT Chemicals & Biochemicals
TRANSCARBOXYLASE
IT Miscellaneous Descriptors
PURIFICATION METHOD
ORGN Super Taxa
Enterobacteriaceae: Eubacteria, Bacteria; Irregular Nonsporing

Gram-Positive Rods: Eubacteria, Bacteria
ORGN Organism Name
irregular nonsporing gram-positive rods (Irregular Nonsporing Gram-Positive Rods); Escherichia coli (Enterobacteriaceae); **Propionibacterium shermanii** (Irregular Nonsporing Gram-Positive Rods)
ORGN Organism Superterms
bacteria; eubacteria; microorganisms
RN 9029-86-1Q (**TRANSCARBOXYLASE**)
9067-78-1Q (**TRANSCARBOXYLASE**)
37340-30-0Q (**TRANSCARBOXYLASE**)

L17 ANSWER 12 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 1993:208135 HCAPLUS
DN 118:208135
TI The nonbiotinylated form of the 1.3 S subunit of **transcarboxylase** binds to avidin (monomeric)-agarose: Purification and separation from the biotinylated 1.3 S subunit
AU Shenoy, Bhami C.; Magner, William J.; Kumar, Ganesh K.; Phillips, Nelson F. B.; Haase, F. Carl; Samols, David
CS Dep. Biochem., Case West. Reserve Univ., Cleveland, OH, 44106-4935, USA
SO Protein Expression and Purification (1993), 4(1), 85-94
CODEN: PEXPEJ; ISSN: 1046-5928
DT Journal
LA English
AB Avidin-biotin technol. is used routinely to purify biotin-contg. carboxylases and also proteins that have been chem. coupled to biotin. The 1.3 S subunit of transcarboxylase (TC) studied here is the biotin-contg. subunit of TC which not only acts as a carboxyl carrier between the CoA ester sites on the central 12 S subunit of TC and keto acid sites on the outer 5 S subunit of TC but also links the 12 S and 5 S subunits together to form a 26 S multisubunit TC complex. The 1.3 S subunit has been cloned, sequenced, and expressed in Escherichia coli. A method for purifying recombinant 1.3 S subunits from E. coli using avidin (monomeric)-agarose column chromatog. has been developed. This affinity-purified 1.3 S was found to be homogeneous by SDS-PAGE, amino acid compn., and N-terminal sequence anal. but had a biotin content of only 28% based on moles of biotin per mol of 1.3 S. This lack of stoichiometry was due to copurifn. of apo-1.3 S as evidenced by the holocarboxylase synthetase reaction. A procedure for sepg. the apo- and biotinylated 1.3 S forms using hydrophobic interaction chromatog. on an Ether 5 PW column is described. The method is based on the difference in hydrophobicity between apo and biotinylated 1.3 S forms. The copurifn. of apo and biotinylated forms of 1.3 S on the avidin (monomeric)-agarose column was found to be due specific interaction with avidin rather than to interaction between apo- and biotinylated 1.3 S forms as demonstrated by the fluorescence quenching studies. The results suggest that the avidin-biotin system by itself may not be sufficient to obtain homogeneous biotinyl proteins as nonbiotinyl protein can also bind avidly to such columns.
IT Protein sequences
(of **transcarboxylase recombinant** 1.3 S subunit biotinylated and nonbiotinylated forms N termini, of **Propionibacterium shermanii**)
IT Molecular association
(of **transcarboxylase recombinant** 1.3 S subunit biotinylated and nonbiotinylated forms of **Propionibacterium shermanii** with avidin, differential basis for)
IT Amino acids, biological studies
RL: BIOL (Biological study)

(of **transcarboxylase recombinant** 1.3 S subunit biotinylated and nonbiotinylated forms, of **Propionibacterium shermanii**)

IT Avidins
RL: BIOL (Biological study)
(**transcarboxylase recombinant** 1.3 S subunit biotinylated and nonbiotinylated forms of **Propionibacterium shermanii** binding by)

IT **Propionibacterium shermanii**
(**transcarboxylase recombinant** 1.3 S subunit biotinylated and nonbiotinylated forms of, avidin binding by and purifn. and sepn. of)

IT Avidins
RL: BIOL (Biological study)
(conjugates, with agarose, **transcarboxylase recombinant** 1.3 S subunit biotinylated and nonbiotinylated forms of **Propionibacterium shermanii** binding by, copurifn. in relation to)

IT 9029-86-1P, **Transcarboxylase**
RL: PREP (Preparation)
(biotinylated and nonbiotinylated forms of 1.3 S subunit of **recombinant**, of **Propionibacterium shermanii**, avidin binding by and purifn. and sepn. of)

IT 58-85-5, Biotin
RL: BIOL (Biological study)
(of **transcarboxylase recombinant** 1.3 S subunit, of **Propionibacterium shermanii**)

IT 9012-36-6D, Agarose, avidin derivs.
RL: BIOL (Biological study)
(**transcarboxylase recombinant** 1.3 S subunit biotinylated and nonbiotinylated forms of **Propionibacterium shermanii** binding by, copurifn. in relation to)

L17 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1993:287164 BIOSIS
DN PREV199345005289
TI **Chimeric** proteins incorporating an in vivo biotinylation domain.
AU Thompson, C. S.; Cress, D. E.
CS Rohm and Haas Co., 727 Norristown Rd., Spring House, PA 19477-0904 USA
SO Protein Engineering, (1993) Vol. 6, No. SUPPL., pp. 70.
Meeting Info.: Winter Symposium on Advances in Gene Technology: Protein Engineering and Beyond Miami, Florida, USA 1993
ISSN: 0269-2139.
DT Conference
LA English
IT Major Concepts
Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics); Genetics; Physiology
IT Chemicals & Biochemicals
TRANSCARBOXYLASE
IT Miscellaneous Descriptors
ABSTRACT; GENETIC ENGINEERING; GENETICALLY ENGINEERED PRODUCT; **TRANSCARBOXYLASE**
ORGN Super Taxa
Enterobacteriaceae: Eubacteria, Bacteria; Irregular Nonsporing Gram-Positive Rods: Eubacteria, Bacteria
ORGN Organism Name
irregular nonsporing gram-positive rods (Irregular Nonsporing Gram-Positive Rods); Escherichia coli (Enterobacteriaceae); **Propionibacterium shermanii** (Irregular Nonsporing

Gram-Positive Rods)
ORGN Organism Superterms
bacteria; eubacteria; microorganisms
RN 9029-86-1Q (**TRANSCARBOXYLASE**)
9067-78-1Q (**TRANSCARBOXYLASE**)
37340-30-0Q (**TRANSCARBOXYLASE**)

L17 ANSWER 14 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 1993:118267 HCAPLUS
DN 118:118267
TI Use of an avidin-binding polypeptide for affinity purification of proteins from transgenic hosts
IN Cress, Dean Ervin; Haase, Ferdinand Carl
PA Rohm and Haas Co., USA
SO Eur. Pat. Appl., 39 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 511747	A1	19921104	EP 1992-303067	19920407
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, PT, SE				
	US 6072039	A	20000606	US 1991-687819	19910419
	CA 2064933	AA	19921020	CA 1992-2064933	19920402
	AU 9213987	A1	19921022	AU 1992-13987	19920402
	AU 659139	B2	19950511		
	NO 9201364	A	19921020	NO 1992-1364	19920408
	BR 9201437	A	19921201	BR 1992-1437	19920416
	JP 06166698	A2	19940614	JP 1992-98307	19920417
PRAI	US 1991-687819		19910419		

AB A peptide that is a substrate for the enzyme biotin holoenzyme synthetase is used in fusion proteins to introduce a site for enzymic biotinylation. The biotinylated protein is then isolated by biotin affinity chromatog. under conditions that avoid the use of denaturants. The peptide is removed from the protein by specific proteolytic or chem. cleavage. An N-terminal domain from the 1.3S subunit of the transcarboxylase of Propionibacterium shermanii was used as the biotin acceptor of a fusion protein. A synthetic gene for a .beta.-endorphin was placed 3' of the sequence encoding the biotinylation domain with the construct connecting the two domains via a labile methionine and the construct expressed in Escherichia coli from the tac promoter. The protein was rapidly purified from cell lysates by chromatog. on an avidin affinity column using acetic acid as the eluant. The fusion protein was cleaved with formic acid to yield .beta.-endorphin or the reverse product depending upon the orientation of the endorphin coding sequence.
IT Avidins
RL: BIOL (Biological study)
(as affinity ligand for purifn. **fusion** proteins biotinylated in vivo)
IT Biotinylation
(enzymic, of **fusion** proteins contg. biotin acceptor domain, for purifn. by avidin affinity chromatog.)
IT Antigens
Enzymes
RL: BIOL (Biological study)
(manuf. in heterologous hosts of, as **fusion** proteins with biotin acceptor domain, purifn. by avidin affinity chromatog. of)
IT Deoxyribonucleic acid sequences
(of genes for .beta.-endorphin analog and biotin-acceptor peptide of

transcarboxylase of human and Propionibacterium)
IT Protein sequences
(of .beta.-endorphin analog and biotin-acceptor peptide of **transcarboxylase** of human and Propionibacterium)
IT Plasmid and Episome
(ptac1.3:endor:1.3:endor, **chimeric** gene for **fusion** protein of .beta.-endorphin and biotin acceptor domain on, expression in Escherichia coli of)
IT Plasmid and Episome
(ptac1.3:endor:endor, **chimeric** gene for **fusion** protein of .beta.-endorphin and biotin acceptor domain on, expression in Escherichia coli of)
IT Plasmid and Episome
(ptac1.3dp:endorB3, **chimeric** gene for **fusion** protein of .beta.-endorphin and biotin acceptor domain on, expression in Escherichia coli of)
IT Plasmid and Episome
(ptac1.3dp:met:endorB3, **chimeric** gene for **fusion** protein of .beta.-endorphin and biotin acceptor domain on, expression in Escherichia coli of)
IT Plasmid and Episome
(ptac1.3dp:revendorB3, **chimeric** gene for **fusion** protein of biotin acceptor domain .beta.-endorphin antisense translation product on, expression in Escherichia coli of)
IT Plasmid and Episome
(ptac:malB:1.3:endorB3, **chimeric** gene for **fusion** protein of .beta.-endorphin and maltose-binding protein and biotin acceptor domain on, expression in Escherichia coli of)
IT Plasmid and Episome
(ptac:malC:endorB3:1.3dp, **chimeric** gene for **fusion** protein of .beta.-endorphin and maltose-binding protein and biotin acceptor domain on, expression in Escherichia coli of)
IT Propionibacterium
(**transcarboxylase** of, biotin-accepting domain of, **fusion** proteins contg., purifn. by avidin affinity chromatog. of)
IT Proteins, specific or class
RL: BIOL (Biological study)
(MBP (maltose-binding protein), **fusion** products, with biotin acceptor domain and .beta.-endorphin, **chimeric** gene for, expression in Escherichia coli of, purifn. by avidin affinity chromatog. of)
IT Proteins, specific or class
RL: BIOL (Biological study)
(**fusion** products, of biotin-accepting domain of **transcarboxylase** and heterologous proteins, manuf. in Escherichia coli of, purifn. by avidin affinity chromatog. of)
IT Gene, animal
RL: BIOL (Biological study)
(synthetic, for .beta.-endorphin of human, in **chimeric** genes for synthesis of **fusion** proteins for purifn. by avidin affinity chromatog.)
IT 39419-81-3
RL: USES (Uses)
(acceptor peptide for, **fusion** proteins contg., for protein purifn. by biotin affinity chromatog.)
IT 58-85-5DP, Biotin, conjugates with **fusion** proteins
RL: PREP (Preparation)
(affinity purifn. of, biotin-accepting domain of **transcarboxylase** in)

IT 66238-14-0
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence of, complete, and manuf. as **fusion** protein with biotin-accepting polypeptide of)
IT 146413-11-8
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence of, complete, **fusion** proteins contg. biotin acceptor domain of)
IT 146413-10-7P
RL: PRP (Properties); PREP (Preparation)
(amino acid sequence of, **fusion** proteins contg., purifn. by avidin affinity chromatog. of)
IT 9013-20-1, Streptavidin
RL: USES (Uses)
(as affinity ligand for purifn. **fusion** proteins biotinylated in vivo)
IT 9029-86-1P, **Transcarboxylase**
RL: PREP (Preparation)
(biotin-accepting peptide of, **fusion** proteins contg., for protein purifn. by biotin affinity chromatog.)
IT 60617-12-1D, .beta.-Endorphin, **fusion** products with biotin acceptor peptide
RL: PRP (Properties); BIOL (Biological study)
(**chimeric** gene for, expression in Escherichia coli of, purifn. by avidin affinity chromatog. of)
IT 98824-75-0, Deoxyribonucleic acid (**Propionibacterium shermanii** methylmalonyl coenzyme A carboxyltransferase biotinyl subunit gene)
RL: PRP (Properties); BIOL (Biological study)
(nucleotide sequence of, complete, **chimeric** genes contg., **fusion** proteins contg. biotin acceptor domain in relation to)
IT 146413-13-0
RL: PRP (Properties); BIOL (Biological study)
(nucleotide sequence of, in **chimeric** genes for prepn. **fusion** protein with biotin-accepting polypeptide)

L17 ANSWER 15 OF 16 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 3
AN 1990-375998 [50] WPIDS
CR 1993-336075 [42]
DNC C1990-163826
TI Post-translation modified **fusion** proteins - providing marker for identifying or isolating **fusion** protein from other materials.
DC B04 D16
IN CRONAN, J E
PA (BIOT-N) BIOTECHNOLOGY RES & DEV CORP; (UNII) UNIV ILLINOIS FOUND; (BIOT-N) BIOTECH R & D CORP; (BIOT-N) BIOTECH R & D CORP
CYC 17
PI WO 9014431 A 19901129 (199050)* 119p
RW: AT BE CH DE DK ES FR GB IT LU NL SE
W: AU CA JP KR
AU 9058270 A 19901218 (199113)
EP 472658 A 19920304 (199210)
R: AT BE CH DE ES FR GB IT LI LU NL SE
JP 04507341 W 19921224 (199306) 30p
AU 647025 B 19940317 (199416)
EP 472658 A4 19920826 (199523)
ADT EP 472658 A EP 1990-909093 19900517; JP 04507341 W JP 1990-508763 19900517, WO 1990-US2852 19900517; AU 647025 B AU 1990-58270 19900517; EP

472658 A4 EP 1990-909093
FDT JP 04507341 W Based on WO 9014431; AU 647025 B Previous Publ. AU 9058270, Based on WO 9014431
PRAI US 1989-354266 19890519
AB WO 9014431 A UPAB: 19991207
The following are claimed: (A) a hybrid DNA sequence encoding a **fusion** protein comprising (a) a first DNA sequence which encodes an amino acid sequence that allows for pos-translational modification of the **fusion** protein and (b) a second DNA sequence joined end to end with the first DNA sequence and in the same reading frame, the second DNA sequencea encoding a selected protein or polypeptide.

The first DNA sequence may code for (i) the 1.3.5 sub unit of **Propionibacterium shermanii transcarboxylase**, tomato biotin protein, the alpha subunit of klebsiella pneumoniae oxalacetate decarboxylase, E. coli biotin carboxyl carrier protein or fragments of these proteins that allow for post-translation biotination of the **fusion** protein or (ii) the dihydrolipoamide acetyltransferase subunit of the E. coli pyruvate dehydrogenase complex or fragments that allow for post-translation lipoylation of the **fusion** protein; (B) a vector comprising a hybrid DNA sequence as in (A) operatively linked to expression control sequences; (C) a host transformed with a vector as in (B); (D) a **fusion** protein comprising a selected protein or polypeptide linked to an amino acid sequence that allows for post-translation modification of the **fusion** protein; the **fusion** protein may be isolated using a binding partner eg. avidin, streptavidin or an organoarsenite.

ADVANTAGE - The post-translation modification provides a marker for the **fusion** protein that can be used, directly or indirectly, to identify the **fusion** protein or to isolate it from a mixt. of other materials, including other proteins.
Dwg.0/0

L17 ANSWER 16 OF 16 HCAPLUS COPYRIGHT 2003 ACS
AN 1989:626534 HCAPLUS
DN 111:226534
TI Expression of synthetic genes fused to biotinyl region of **transcarboxylase of Propionibacterium shermanii** in Escherichia coli. Attempt of in vivo biotinylation to facilitate protein purification
AU Sato, Naoko; Kojima, Hiroyuki
CS Govern Ind. Res. Inst., Osaka, Japan
SO Osaka Kogyo Gijutsu Shikensho Kiho (1989), 40(2), 76-86
CODEN: OKGKAE; ISSN: 0472-142X
DT Journal
LA Japanese
AB In vivo biotinylation with synthetic genes was studied in order to facilitate purifn. of a recombinant gene product, based on specific affinity of biotin to avidin. A partial DNA sequence of Propionibacterium shermanii transcarboxylase 1.3.5 biotinyl subunit was chosen for the biotinylation (biotin-tail, BT), including an evolutionarily conserved structure of biotin enzymes from the tetrapeptides of biotinylation site to the carboxyl terminal. Three expression vectors were constructed: the vector pDR-BT was directed to express only BT protein, pUC-BT to express a fusion protein of a part of .beta.-galactosidase and BT, and pDRCm-BT to express a fusion protein of chloramphenicol acetyltransferase and BT. Their expression products in Escherichia coli were analyzed by SDS-PAGE and fluorog. with 14C-biotin. No vector produced a biotinylated protein, although pUC-BT and pDRCm-BT produced fused proteins as expected. The failure of biotinylation is discussed.
IT Molecular cloning

(of **transcarboxylase** gene, of **Propionibacterium shermanii**, in Escherichia coli, biotinylation in)

IT Escherichia coli
(**recombinant** protein purifn. from, biotinylation in)

IT **Propionibacterium shermanii**
(**transcarboxylase** gene of, purifn. of **recombinant**, biotinylation in)

IT 37340-30-0P, **Transcarboxylase**
RL: PUR (Purification or recovery); PREP (Preparation)
(purifn. of **recombinant**, biotinylation in)

IT 58-85-5, Biotin
RL: RCT (Reactant); RACT (Reactant or reagent)
(**recombinant** protein purifn. by complexing with)

```
=> fil reg
FILE 'REGISTRY' ENTERED AT 13:15:27 ON 01 MAY 2003
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
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DICTIONARY FILE UPDATES:  30 APR 2003  HIGHEST RN 508165-25-1

TSCA INFORMATION NOW CURRENT THROUGH JANUARY 6, 2003

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

Crossover limits have been increased.  See HELP CROSSOVER for details.

Experimental and calculated property data are now available.  See HELP
PROPERTIES for more information.  See STNote 27, Searching Properties
in the CAS Registry File, for complete details:
http://www.cas.org/ONLINE/STN/STNOTES/stnotes27.pdf

=> d que l1
L1            15 SEA FILE=REGISTRY ABB=ON  PLU=ON  KLKVTVNGTAYDVDVDVDKSHEN|PAPLA
                 GTVSKILV.*VKERDAVQGGQGL/SQSP
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L> seq ID 1 + 2

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=> d l1 sqide 1-15

L1   ANSWER 1 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   503078-37-3  REGISTRY
CN   INDEX NAME NOT YET ASSIGNED
FS   PROTEIN SEQUENCE
SQL  123

SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PRAAGGAGAG
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        51 KAGEGEIPAP LAGTVSKILV KEGDTVKAGQ TVLVLEAMKM ETEINAPTDG
                  === ========== ========== ========== ==========
       101 KVEKVLVKER DAVQGGQGLI KIG
           ========== =========
HITS AT:   2-24, 58-119

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS
                1 REFERENCES IN FILE CA (1957 TO DATE)
                1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1   ANSWER 2 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   503078-30-6  REGISTRY
CN   INDEX NAME NOT YET ASSIGNED
FS   PROTEIN SEQUENCE
SQL  133

SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
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         51 AGEGEIPAPL AGTVSKILVK EGDTVKAGQT VLVLEAMKME TEINAPTDGK
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        101 VEKVLVKERD AVQGGQGLIK IGDLELIEGR EKL
            ========== ========
HITS AT:   2-24, 57-118
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS
                1 REFERENCES IN FILE CA (1957 TO DATE)
                1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1   ANSWER 3 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   488278-18-8  REGISTRY
CN   GenBank AAA25674 (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AAA25674 (Translated from: GenBank M11738)
FS   PROTEIN SEQUENCE
SQL  123

SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PRAAGGAGAG
            ========= ========== ====
         51 KAGEGEIPAP LAGTVSKILV KEGDTVKAGQ TVLVLEAMKM ETEINAPTDG
                 === ========== ========== ========== ==========
        101 KVEKVLVKER DAVQGGQGLI KIG
            ========== =========
HITS AT:   2-24, 58-119

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**
MF   Unspecified
CI   MAN
SR   GenBank

L1   ANSWER 4 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   480043-41-2  REGISTRY
CN   GenBank AAA89094 (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AAA89094 (Translated from: GenBank U47628)
FS   PROTEIN SEQUENCE
SQL  129

SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
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                 ==== ========== ========== ========== ==========
        101 VEKVLVKERD AVQGGQGLIK IGDLELIEG
            ========== ========
HITS AT:   2-24, 57-118

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**
MF   Unspecified
CI   MAN
SR   GenBank

L1   ANSWER 5 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   480043-39-8  REGISTRY
CN   GenBank AAA89092 (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AAA89092 (Translated from: GenBank U47627)
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FS   PROTEIN SEQUENCE
SQL  129

SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
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        51 AGEGEIPAPL AGTVSKILVK EGDTVKAGQT VLVLEAMKME TEINAPTDGK
              ==== ========== ========== ========== ==========
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           ========== ========
HITS AT:   2-24, 57-118

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**
MF   Unspecified
CI   MAN
SR   GenBank

L1   ANSWER 6 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   480043-37-6  REGISTRY
CN   GenBank AAA89090 (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AAA89090 (Translated from: GenBank U47626)
FS   PROTEIN SEQUENCE
SQL  129

SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
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              ==== ========== ========== ========== ==========
       101 VEKVLVKERD AVQGGQGLIK IGDLELIEG
           ========== ========
HITS AT:   2-24, 57-118

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**
MF   Unspecified
CI   MAN
SR   GenBank

L1   ANSWER 7 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   466712-46-9  REGISTRY
CN   2: PN: US20020142355 SEQID: 2 unclaimed protein (9CI)  (CA INDEX NAME)
FS   PROTEIN SEQUENCE
SQL  70

PATENT ANNOTATIONS (PNTE):
Sequence |Patent
Source   |Reference
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Not Given|US2002142355
         |unclaimed
         |SEQID 2


SEQ      1 EGEIPAPLAG TVSKILVKEG DTVKAGQTVL VLEAMKMETE INAPTDGKVE
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        51 KVLVKERDAV QGGQGLIKIG
           ========== ======
HITS AT:   5-66
MF   Unspecified
CI   MAN
SR   CA
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LC   STN Files:   CA, CAPLUS, USPATFULL
                  1 REFERENCES IN FILE CA (1957 TO DATE)
                  1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1   ANSWER 8 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   466701-20-2  REGISTRY
CN   1: PN: US20020142355 SEQID: 1 claimed protein (9CI)  (CA INDEX NAME)
FS   PROTEIN SEQUENCE
SQL  122

PATENT ANNOTATIONS (PNTE):
Sequence |Patent
Source   |Reference
=========+============
Not Given|US2002142355
         |claimed
         |SEQID 1


SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
            ========= ========== ====
        51 AGEGEIPAPL AGTVSKILVK EGDTVKAGQT VLVLEAMKME TEINAPTDGK
               ==== ========== ========== ========== ==========
       101 VEKVLVKERD AVQGGQGLIK IG
           ========== ========
HITS AT:   2-24, 57-118
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
                  1 REFERENCES IN FILE CA (1957 TO DATE)
                  1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1   ANSWER 9 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   261151-38-6  REGISTRY
CN   Protein (synthetic biotin purification tag) fusion protein with antigen
     (Treponema pallidium 17-kilodalton) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   6: PN: EP985931 SEQID: 7 claimed protein
FS   PROTEIN SEQUENCE
SQL  266

PATENT ANNOTATIONS (PNTE):
Sequence |Patent
Source   |Reference
=========+==========
Not Given|EP985931
         |claimed
         |SEQID 7


SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
            ========= ========== ====
        51 AGEGEIPAPL AGTVSKILVK EGDTVKAGQT VLVLEAMKME TEINAPTDGK
               ==== ========== ========== ========== ==========
       101 VEKVLVKERD AVQGGQGLIK IGDLELIEGR EKLSCTTVCP HAGKAKAEKV
           ========== ========
       151 ECALKGGIFR GTLPAADCPG IDTTVTFNAD GTAQKVELAL EKKSAPSPLT
       201 YRGTWMVRED GIVELSLVSS EQSKAPHEKE LYELIDSNSV RYMGAPGAGK
       251 PSKEMAPFYV LKKTKK
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HITS AT: 2-24, 57-118
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS
1 REFERENCES IN FILE CA (1957 TO DATE)
1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1 ANSWER 10 OF 15 REGISTRY COPYRIGHT 2003 ACS
RN 261151-36-4 REGISTRY
CN Protein (synthetic biotin purification tag) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 4: PN: EP985931 SEQID: 5 claimed protein
FS PROTEIN SEQUENCE
SQL 126

PATENT ANNOTATIONS (PNTE):

Sequence Source	Patent Reference
Not Given	EP985931 claimed SEQID 5

SEQ 1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
51 AGEGEIPAPL AGTVSKILVK EGDTVKAGQT VLVLEAMKME TEINAPTDGK
101 VEKVLVKERD AVQGGQGLIK IGDLEL

HITS AT: 2-24, 57-118
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS
1 REFERENCES IN FILE CA (1957 TO DATE)
1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1 ANSWER 11 OF 15 REGISTRY COPYRIGHT 2003 ACS
RN 261151-34-2 REGISTRY
CN Protein (synthetic biotin purification tag) fusion protein with antigen (Treponema pallidium 15-kilodalton) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 2: PN: EP985931 SEQID: 3 claimed protein
FS PROTEIN SEQUENCE
SQL 256

PATENT ANNOTATIONS (PNTE):

Sequence Source	Patent Reference
Not Given	EP985931 claimed SEQID 3

SEQ 1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
51 AGEGEIPAPL AGTVSKILVK EGDTVKAGQT VLVLEAMKME TEINAPTDGK

```
                ==== ========== ========== ========== ==========
       101 VEKVLVKERD AVQGGQGLIK IGDLELIEGR EKLSFSSIPN GTYRATYQDF
           ========== ========
       151 DENGWKDFLE VTFDGGKMVQ VVYDYQHKEG RFKSQDADYH RVMYASSGIG
       201 PEKAFRELAD ALLEKGNPEM VDVVTGATVS SQSFRRLGRA LLQSARRGEK
       251 EAIISR
HITS AT:   2-24, 57-118
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS
                1 REFERENCES IN FILE CA (1957 TO DATE)
                1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1   ANSWER 12 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   257260-46-1  REGISTRY
CN   Protein (Borrelia burgdorferi strain S-1-10 gene OspC 194-amino acid
     fragment) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   92: PN: WO0005378 SEQID: 2 claimed protein
FS   PROTEIN SEQUENCE
SQL  194

PATENT ANNOTATIONS (PNTE):
Sequence |Patent
Source   |Reference
=========+============
Not Given|WO2000005378
         |claimed
         |SEQID 2


SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PAAGGAGAGK
           ========= ========== ====
        51 AGEGEIPAPL AGTVSKILVK EGDTVKAGQT VLVLEAMKME TEINAPTDGK
                ==== ========== ========== ========== ==========
       101 VEKVLVKERD AVQGGQGLIK IGDLELIEGR ESFSWDPVPI SDLPKTHNTK
           ========== ========
       151 DKGAEELVKL AESVAGLLKV AQETLNNSVK ELTSPVVAES PKKP
HITS AT:   2-24, 57-118
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
                1 REFERENCES IN FILE CA (1957 TO DATE)
                1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1   ANSWER 13 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   134801-98-2  REGISTRY
CN   49-123-Carboxyltransferase, methylmalonyl coenzyme A (Propionibacterium
     shermanii biotinyl subunit) (9CI)  (CA INDEX NAME)
FS   PROTEIN SEQUENCE
SQL  75

SEQ      1 AGKAGEGEIP APLAGTVSKI LVKEGDTVKA GQTVLVLEAM KMETEINAPT
                   = ========== ========== ========== ==========
        51 DGKVEKVLVK ERDAVQGGQG LIKIG
           ========== ========== =
HITS AT:   10-71
MF   C331 H568 N90 O107 S2
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CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
               1 REFERENCES IN FILE CA (1957 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1   ANSWER 14 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   134774-01-9  REGISTRY
CN   18-123-Carboxyltransferase, methylmalonyl coenzyme A (Propionibacterium
     shermanii biotinyl subunit) (9CI)  (CA INDEX NAME)
FS   PROTEIN SEQUENCE
SQL  106

SEQ      1 VDKSHENPMG TILFGGGTGG APAPRAAGGA GAGKAGEGEI PAPLAGTVSK
                                                     ==========
        51 ILVKEGDTVK AGQTVLVLEA MKMETEINAP TDGKVEKVLV KERDAVQGGQ
           ========== ========== ========== ========== ==========
       101 GLIKIG
           ==
HITS AT:   41-102
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
               1 REFERENCES IN FILE CA (1957 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1957 TO DATE)

L1   ANSWER 15 OF 15  REGISTRY  COPYRIGHT 2003 ACS
RN   72103-05-0  REGISTRY
CN   Carboxyltransferase, methylmalonyl coenzyme A (Propionibacterium shermanii
     biotinyl subunit) (9CI)  (CA INDEX NAME)
FS   PROTEIN SEQUENCE
SQL  123
NTE  modified (modifications unspecified)

SEQ      1 MKLKVTVNGT AYDVDVDVDK SHENPMGTIL FGGGTGGAPA PRAAGGAGAG
           ========= ========== ====
        51 KAGEGEIPAP LAGTVSKILV KEGDTVKAGQ TVLVLEAMKM ETEINAPTDG
              === ========== ========== ========== ==========
       101 KVEKVLVKER DAVQGGQGLI KIG
           ========== =========
HITS AT:   2-24, 58-119

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**
DR   146413-11-8
MF   Unspecified
CI   MAN
LC   STN Files:   CA, CAPLUS, USPATFULL
               2 REFERENCES IN FILE CA (1957 TO DATE)
               2 REFERENCES IN FILE CAPLUS (1957 TO DATE)


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FILE COVERS 1907 - 1 May 2003 VOL 138 ISS 18
FILE LAST UPDATED: 30 Apr 2003 (20030430/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

'OBI' IS DEFAULT SEARCH FIELD FOR 'HCAPLUS' FILE

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L1 15 SEA FILE=REGISTRY ABB=ON PLU=ON KLKVTVNGTAYDVDVDVDKSHEN|PAPLA GTVSKILV.*VKERDAVQGGQGL/SQSP
L2 8 SEA FILE=HCAPLUS ABB=ON PLU=ON L1

=> d :ca 1-8 l2

L2 ANSWER 1 OF 8 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:238328 HCAPLUS
DOCUMENT NUMBER: 138:268069
TITLE: Method for an in vitro sequence specific biotinylation of polypeptides
INVENTOR(S): Ambrosius, Dorothee; Lanzendoerfer, Martin; Schraeml, Michael; Watzele, Manfred
PATENT ASSIGNEE(S): F. Hoffmann-La Roche AG, Switz.
SOURCE: Eur. Pat. Appl., 19 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1295894	A1	20030326	EP 2002-21322	20020920

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

PRIORITY APPLN. INFO.: EP 2001-122554 A 20010925
EP 2001-129681 A 20011213

AB A method of prepg. a biotinylated polypeptide in a cell-free peptide synthesis reaction mixt. which contains ribosomes, tRNA, ATP, GTP, nucleotides and amino acids, characterized in that (a) a nucleic acid is expressed to form said polypeptide which contains a holocarboxylase synthetase (BirA) substrate sequence tagged at either end; (b) said polypeptide is biotinylated in the presence of biotin and BirA; (c) said biotinylated polypeptide is isolated from said mixt.; or said mixt. is incubated with immobilized avidin or streptavidin under such conditions that said biotinylated polypeptide is bound to said immobilized avidin or streptavidin.

IC ICM C07K001-13
CC 9-16 (Biochemical Methods)
Section cross-reference(s): 6, 7

IT **503078-30-6 503078-37-3 503078-38-4**
RL: PRP (Properties)
(unclaimed protein sequence; method for an in vitro sequence specific biotinylation of polypeptides)
REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 8 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:755073 HCAPLUS
DOCUMENT NUMBER: 137:275370
TITLE: Methods for the in vivo biotin labeling of polypeptides
INVENTOR(S): Barry, Michael A.; Parrott, Michael B.
PATENT ASSIGNEE(S): Baylor College of Medicine, USA
SOURCE: U.S. Pat. Appl. Publ., 13 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002142355	A1	20021003	US 2001-987485	20011114

PRIORITY APPLN. INFO.: US 2000-247965P P 20001114
AB The present invention is directed to methods that can used for biotin labeling polypeptides in mammalian cells. The methods can be effectively used for cytoplasmic proteins, secreted proteins, and for proteins found on viral surfaces.
IC ICM G01N033-53
ICS C12P021-06; C07H021-04; C12N009-00; C12P021-02; C12N005-06; C12N015-00; C12N015-09; C12N015-63; C12N015-70; C12N015-74; C12N005-00; C12N005-02
NCL 435007500
CC 9-16 (Biochemical Methods)
IT **466701-20-2**
RL: BSU (Biological study, unclassified); RCT (Reactant); BIOL (Biological study); RACT (Reactant or reagent)
(amino acid sequence; methods for in vivo biotin labeling of polypeptides)
IT **466712-46-9**
RL: PRP (Properties)
(unclaimed protein sequence; methods for the in vivo biotin labeling of polypeptides)

L2 ANSWER 3 OF 8 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:175590 HCAPLUS
DOCUMENT NUMBER: 132:219217
TITLE: Recombinant antigen immunoassay for the diagnosis of syphilis
INVENTOR(S): Mullenix, Michael C.; Deutsch, John
PATENT ASSIGNEE(S): Becton, Dickinson and Company, USA
SOURCE: Eur. Pat. Appl., 16 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

EP 985931	A2	20000315	EP 1999-115877	19990812
EP 985931	A3	20000329		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.: US 1998-148920 19980904

AB A method for detecting anti-Treponema pallidum antibody and diagnosing syphilis has been provided. Fusion protein antigens from the fusion of a peptide sequence having an amino acid sequence encoded by the described nucleic acid sequence to Treponema pallidum membrane proteins are used as antigens in immunoassay of test samples for the presence of anti-Treponema pallidum membrane protein antibodies. A test kit for diagnosing syphilis is also provided comprising a container having therein the fusion protein antigens.

IC ICM G01N033-571
ICS G01N033-543; G01N033-558; C12N015-30; C07K014-44

CC 9-10 (Biochemical Methods)
Section cross-reference(s): 3, 14, 15

IT **261151-34-2 261151-36-4 261151-38-6**
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; recombinant antigen immunoassay for diagnosis of syphilis)

L2 ANSWER 4 OF 8 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:98788 HCAPLUS
DOCUMENT NUMBER: 132:150602
TITLE: Uses of borreliacidal epitopes of Borrelia burgdorferi outer surface protein C (OspC) in vaccines
INVENTOR(S): Callister, Steven N.; Lovrich, Steven D.; Schell, Ronald F.; Jobe, Dean A.
PATENT ASSIGNEE(S): Gundersen Lutheran Medical Foundation, Inc., USA
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000006745	A1	20000210	WO 1999-US17270	19990730

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9953282	A1	20000221	AU 1999-53282	19990730
US 6210676	B1	20010403	US 1999-364083	19990730
EP 1100922	A1	20010523	EP 1999-938897	19990730

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

JP 2002523019	T2	20020730	JP 2000-562527	19990730
US 6464985	B1	20021015	US 2000-651419	20000830
NO 2001000412	A	20010329	NO 2001-412	20010124

PRIORITY APPLN. INFO.: US 1998-94955P P 19980731

US 1999-364083 A3 19990730
WO 1999-US17270 W 19990730

AB An epitope of the outer membrane protein C (OspC) of Borrelia burgdorferi is described for the prevention, treatment and early diagnosis of Lyme disease in humans and other animals. This invention also relates to a screening method detecting anti-Osp borreliacidal antibody activity, and antibodies reacting with a protein fragment encoded by a DraI-SmaI DNA fragment of the OspC gene. The OspC gene was cloned and deletion anal. was used to identify the region of the gene encoding a borreliacidal epitope. Borreliacidal antibodies to OspC were found in the serum of early Lyme disease patients and absorption of the serum with the OspC epitope lowered the borreliacidal activity 32-512-fold.

IC ICM C12N015-31
ICS C07K014-20; A61K039-02; G01N033-569

CC 15-2 (Immunochemistry)
Section cross-reference(s): 3, 10

IT **257260-46-1**
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(amino acid sequence; uses of borreliacidal epitopes of Borrelia burgdorferi outer surface protein C (OspC) in vaccines)

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 5 OF 8 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:118267 HCAPLUS
DOCUMENT NUMBER: 118:118267
TITLE: Use of an avidin-binding polypeptide for affinity purification of proteins from transgenic hosts
INVENTOR(S): Cress, Dean Ervin; Haase, Ferdinand Carl
PATENT ASSIGNEE(S): Rohm and Haas Co., USA
SOURCE: Eur. Pat. Appl., 39 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 511747	A1	19921104	EP 1992-303067	19920407
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, PT, SE				
US 6072039	A	20000606	US 1991-687819	19910419
CA 2064933	AA	19921020	CA 1992-2064933	19920402
AU 9213987	A1	19921022	AU 1992-13987	19920402
AU 659139	B2	19950511		
NO 9201364	A	19921020	NO 1992-1364	19920408
BR 9201437	A	19921201	BR 1992-1437	19920416
JP 06166698	A2	19940614	JP 1992-98307	19920417

PRIORITY APPLN. INFO.: US 1991-687819 19910419

AB A peptide that is a substrate for the enzyme biotin holoenzyme synthetase is used in fusion proteins to introduce a site for enzymic biotinylation. The biotinylated protein is then isolated by biotin affinity chromatog. under conditions that avoid the use of denaturants. The peptide is removed from the protein by specific proteolytic or chem. cleavage. An N-terminal domain from the 1.3S subunit of the transcarboxylase of Propionibacterium shermanii was used as the biotin acceptor of a fusion protein. A synthetic gene for a .beta.-endorphin was placed 3' of the sequence encoding the biotinylation domain with the construct connecting

the two domains via a labile methionine and the construct expressed in Escherichia coli from the tac promoter. The protein was rapidly purified from cell lysates by chromatog. on an avidin affinity column using acetic acid as the eluant. The fusion protein was cleaved with formic acid to yield .beta.-endorphin or the reverse product depending upon the orientation of the endorphin coding sequence.

IC ICM C12N015-62
ICS C07K003-18; C07K013-00
ICA C12N005-00
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): 16
IT **146413-11-8**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence of, complete, fusion proteins contg. biotin acceptor domain of)

L2 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1991:443476 HCAPLUS
DOCUMENT NUMBER: 115:43476
TITLE: Fusion proteins having an in vivo post-translational modification site and methods of manufacture and purification
INVENTOR(S): Cronan, John E., Jr.
PATENT ASSIGNEE(S): Biotechnology Research and Development Corp., Inc., USA; University of Illinois
SOURCE: PCT Int. Appl., 120 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9014431	A1	19901129	WO 1990-US2852	19900517
W: AU, CA, JP, KR				
RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE				
CA 2057908	AA	19901120	CA 1990-2057908	19900517
AU 9058270	A1	19901218	AU 1990-58270	19900517
AU 647025	B2	19940317		
EP 472658	A1	19920304	EP 1990-909093	19900517
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
JP 04507341	T2	19921224	JP 1990-508763	19900517
US 5252466	A	19931012	US 1990-525568	19900518
PRIORITY APPLN. INFO.:			US 1989-354266	19890519
			WO 1990-US2852	19900517

AB Recombinant proteins contg. a posttranslational modification site, i.e. a site for biotinylation or lipoylation, are prepd. The posttranslational modification aids in purifn. of the fusion proteins. Plasmids contg. chimeric genes for .beta.-galactosidase fused to biotinylation sites of Escherichia coli biotin carboxyl carrier protein, the 1.3S subunit of Propionibacterium shermanii transcarboxylase or a tomato sequence homologous to this protein, human pyruvate carboxylase, or Saccharomyces cerevisiae pyruvate carboxylase were prepd. E. coli transformed with these plasmids produced biotinylated fusion proteins which were purified on low-affinity "monomer avidin" columns (Sigma Chem. Co.). Fusion proteins contg. lipoylation site(s) of the E2p subunit of E. coli pyruvate dehydrogenase were similarly produced and purified with a p-aminophenylarsine oxide-Sepharose column.

IC ICM C12P021-00
ICS C12N015-00; C07K003-18; C07K013-00
CC 3-4 (Biochemical Genetics)
IT **134774-01-9 134801-98-2**
RL: PRP (Properties)
(transcarboxylase biotinylation site, recombinant fusion proteins contg., protein purifn. in relation to)

L2 ANSWER 7 OF 8 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1985:573361 HCAPLUS
DOCUMENT NUMBER: 103:173361
TITLE: Cloning and expression of the 1.3 S biotin-containing subunit of transcarboxylase
AUTHOR(S): Murtif, Vicki L.; Bahler, Chris R.; Samols, David
CORPORATE SOURCE: Dep. Biochem., Case Western Reserve Univ., Cleveland, OH, 44106, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1985), 82(17), 5617-21
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene coding for the 1.3 S biotin-contg. subunit of transcarboxylase (EC 2.1.3.1) [9029-86-1] from Propionibacterium shermanii was cloned. Transcarboxylase is a well-characterized enzyme composed of 30 polypeptides of 3 different types; 12 1.3 S biotinyl subunits, 6 5 S dimeric outer subunits, and 1 12 S hexameric central subunit. In propionic acid fermn., the enzyme catalyzes the transfer of a carboxyl group from methylmalonyl-CoA to pyruvate in 2 partial reactions. The 1.3 S subunit binds the outer and central subunits of the enzyme together, and its biotin serves as carboxyl carrier between subsites on the central and outer subunits where each partial reaction occurs. The cloned gene was expressed in Escherichia coli, and the 1.3 S subunit accumulates to 7% of total cellular protein. The foreign protein is recognized and biotinated by biotin holoenzyme synthetase of E. coli. The identifications of the gene and its product were confirmed by 4 independent approaches; DNA sequence anal., immunopptn., incorporation of labeled biotin, and measurement of enzymic activity in the 1st partial reaction.
CC 3-4 (Biochemical Genetics)
Section cross-reference(s): 10
IT **72103-05-0 98824-75-0**
RL: PRP (Properties); BIOL (Biological study)
(nucleotide sequence of)

L2 ANSWER 8 OF 8 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1980:2376 HCAPLUS
DOCUMENT NUMBER: 92:2376
TITLE: Amino acid sequence of the biotinyl subunit from transcarboxylase
AUTHOR(S): Maloy, W. Lee; Bowien, Botho U.; Zwolinski, Gene K.; Kumar, K. Ganesh; Wood, Harland G.; Ericsson, Lowell H.; Walsh, Kenneth A.
CORPORATE SOURCE: Sch. Med., Case West. Reserve Univ., Cleveland, OH, 44106, USA
SOURCE: Journal of Biological Chemistry (1979), 254(22), 11615-22
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The complete amino acid sequence of the biotinyl subunit of transcarboxylase of Propionibacterium shermanii was detd. from the

structures of overlapping tryptic and CNBr peptides together with sequenator anal. on the whole subunit. The subunit contained 123 amino acid residues. Eleven of 19 residues in the region of biotin attachment, when compared to pyruvate carboxylase from avian liver, were in identical positions relative to biocytin (Bct). There was less homol. with acetyl-CoA carboxylase from Escherichia coli, but in all of these biotin enzymes there was an Ala-Met-Bct-Met sequence. The secondary structure of the biotinyl subunit was estd. and considered in relation to the role of the biotinyl subunit in the structure and function of transcarboxylase.

CC 7-5 (Enzymes)

IT **72103-05-0**

RL: PRP (Properties); BIOL (Biological study)
(amino acid sequence of)

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=> d que l7
L1          1203 SEA BIOTIN? (L) PEPTIDE#
L2           133 SEA TRANSCARBOXYLASE? OR CARBOXYTRANSFERAS?
L3             5 SEA L1 AND L2
L4          4158 SEA BIOTINYLAT?
L5          1189 SEA L4 (L) (PEPTIDE? OR PROTEIN?)
L6            14 SEA L5 AND L2
L7            17 SEA L6 OR L3


=> d bib ab 1-17

L7   ANSWER 1 OF 17  HCAPLUS  COPYRIGHT 2003 ACS
AN   2002:199169  HCAPLUS
DN   137:75477
TI   Metabolic biotinylation of recombinant proteins in
     mammalian cells and in mice
AU   Parrott, M. Brandon; Barry, Michael A.
CS   Department of Microbiology and Immunology, Baylor College of Medicine,
     Houston, TX, 77030, USA
SO   Molecular Therapy (2000), 1(1), 96-104
     CODEN: MTOHCK; ISSN: 1525-0016
PB   Academic Press
DT   Journal
LA   English
AB   The avidin-biotin system is a fundamental technol. in biomedicine for
     immunolocalization, imaging, nucleic acid blotting, and protein labeling.
     While this technol. is robust, it is limited by the fact that mammalian
     proteins must be expressed and purified prior to chem. biotinylation using
     crosslinking agents which modify proteins at random locations to
     heterogeneous levels and can inactivate protein function.  To circumvent
     this limitation, we demonstrate the ability to metabolically biotinylate
     tagged proteins in mammalian cells and in mice using the endogenous
     biotinylation enzymes of the host.  Endogenously biotinylated proteins
     were readily purified from mammalian cells using monomeric avidin and
     eluted under nondenaturing conditions using only biotin as the releasing
     agent.  This technol. should allow recombinant proteins and fragile
     protein complexes to be produced and purified from mammalian cells as well
     as from transgenic plants and animals.  In addn., this technol. may be
     particularly useful for cell-targeting applications in which proteins or
     viral gene therapy vectors can be biotinylated at genetically defined
     sites for combination with other targeting moieties complexed with avidin.
     (c) 2000 Academic Press.
RE.CNT  23    THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7   ANSWER 2 OF 17  HCAPLUS  COPYRIGHT 2003 ACS
AN   2001:164286  HCAPLUS
DN   134:292368
TI   Metabolic biotinylation of secreted and cell surface
     proteins from mammalian cells
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AU Parrott, M. Brandon; Barry, Michael A.
CS Department of Immunology, Baylor College of Medicine, Houston, TX, USA
SO Biochemical and Biophysical Research Communications (2001), 281(4), 993-1000
CODEN: BBRCA9; ISSN: 0006-291X
PB Academic Press
DT Journal
LA English
AB Due to its strength and specificity, the interaction between avidin and biotin has been used in a variety of medical and scientific applications ranging from drug targeting to immunohistochem. To maximize the application of this technol. in mammalian systems, we recently demonstrated the ability to metabolically biotinylate tagged proteins in mammalian cells using the endogenous biotin ligase enzymes of the mammalian cell. This technol. allows site-specific biotinylation without any exogenous reagents and eliminates possible inactivation of the protein of interest by nonspecific biotinylation. Here, we report further expansion of the mammalian metabolic biotinylation technol. to enable biotinylation of proteins secreted from mammalian cells and expressed on their cell surface by cosecretion with BirA, the biotin ligase of E. coli. This technique can be used to biotinylate secreted proteins for purifn. or targeting and also for biotinylating the surfaces of mammalian cells to facilitate their labeling and purifn. from other nontagged cells. (c) 2001 Academic Press.
RE.CNT 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1999:608309 HCAPLUS
DN 132:1507
TI Expression and **Biotinylation** of a Mutant of the **Transcarboxylase** Carrier **Protein** from Propioni shermanii
AU Jank, Matthias M.; Bokorny, Stefan; Rohm, Klaus - Heinrich; Berger, Stefan
CS Institut fur Analytische Chemie, Universitat Leipzig, Leipzig, 04103, Germany
SO Protein Expression and Purification (1999), 17(1), 123-127
CODEN: PEXPEJ; ISSN: 1046-5928
PB Academic Press
DT Journal
LA English
AB A deletion mutant (residues 10 to 48 cut) of the biotinyl subunit (tcc) from the enzyme transcarboxylase (EC 2.1.3.1) of Propioni shermanii was over-expressed in Escherichia coli. Complete biotinylation of the protein was achieved by addn. of exogenous biotin and co-expression of the biotin holoenzyme synthetase (EC 6.3.4.15.) from E. coli. The transcription of both genes was put under control of different operators/promoters, thus achieving independent control of expression levels and optimized yields of the holo-tcc. Bacteria were grown in a biotin-supplemented minimal medium (M9) that contained [13C]glucose as the carbon source and [15N]NH4Cl as the sole nitrogen source. The target protein could be purified to homogeneity by ion-exchange chromatog. and concd. to NMR-suitable concns. (2 mM) without aggregation. (c) 1999 Academic Press.
RE.CNT 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1998:640407 HCAPLUS
DN 129:272665
TI High throughput assays using fusion proteins for screening binding compounds and protease inhibitors

IN Hermes, Jeffrey D.; Salowe, Scott P.; Sinclair, Peter J.
PA Merck & Co., Inc., USA
SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9841866	A1	19980924	WO 1998-US4610	19980310

W: CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRAI US 1997-40795P P 19970314

AB This application describes a high throughput assay for screening compds. which are capable of binding to a fusion protein which consists of a target protein and an FK506-binding protein. This application also describes an assay for screening compds. which inhibit a protease. A FK506-binding protein-ZAP70 tandem SH2 domains fusion protein was recombinantly prepd., expressed in Escherichia coli, and purified by affinity chromatog. on agarose-immobilized avidin having bound biotinylated phosphopeptide derived from the .zeta.1 ITAM sequence of the human T-cell receptor. Inhibitors of the fusion protein are screened using the biotinylphosphopeptide, the fusion protein, and europium cryptate-labeled FK506 analog in wells of a 96-well black microplate. The fluorescence ratio is measured in a Packard Discovery homogeneous time-resolved fluorescence analyzer.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1996:541213 HCAPLUS
DN 125:189979
TI Method for preparing scintillation proximity assay targets
IN Lerner, Claude G.
PA Abbott Laboratories, USA
SO PCT Int. Appl., 40 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9621156	A1	19960711	WO 1995-US16736	19951219

W: CA, JP, MX
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRAI US 1995-368467 19950104

AB A method is disclosed for immobilizing an assay target on a fluorescent support for use in a scintillation proximity assay, comprising the steps of (1) expressing a fusion protein comprising a linking domain and a functional domain, and (2) attaching said fusion protein to said fluorescent support via said linking domain, wherein the functional domain comprises the assay target or a polypeptide capable of attachment to the assay target.

L7 ANSWER 6 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1996:326543 HCAPLUS
DN 125:29330
TI **Biotinylation** in vivo as a sensitive indicator of **protein** secretion and membrane **protein** insertion
AU Jander, Georg; Cronan, John E., Jr.; Beckwith, Jon

CS Dep. Microbiology Molecular Genetics, Harvard Med. Sch., Boston, MA, 02115, USA
SO Journal of Bacteriology (1996), 178(11), 3049-3058
CODEN: JOBAAY; ISSN: 0021-9193
PB American Society for Microbiology
DT Journal
LA English
AB Escherichia coli biotin ligase is a cytoplasmic protein which specifically biotinylates the biotin-accepting domains from a variety of organisms. This in vivo biotinylation can be used as a sensitive signal to study protein secretion and membrane protein insertion. When the biotin-accepting domain from the 1.3S subunit of Propionibacterium shermanii transcarboxylase (PSBT) is translationally fused to the periplasmic proteins alk. phosphatase and maltose-binding protein, there is little or no biotinylation of PSBT in wild-type E. coli. Inhibition of SecA with NaN3 and mutations in SecB, SecD, and SecF, all of which slow down protein secretion, result in biotinylation of PSBT. When PSBT is fused to the E. coli inner membrane protein MalF, it acts as a topol. marker: fusions to cytoplasmic domains of MalF are biotinylated, and fusions to periplasmic domains are generally not biotinylated. If SecA is inhibited by NaN3 or if the SecE in the cell is depleted, then the insertion of the MalF 2nd periplasmic domain is slowed down enough that PSBT fusions in this part of the protein become biotinylated. Compared with other protein fusions that have been used to study protein translocation, PSBT fusions have the advantage that they can be used to study the rate of the insertion process.

L7 ANSWER 7 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1993:442166 HCAPLUS
DN 119:42166
TI In vivo **biotinylation** of fusion **proteins** expressed in Escherichia coli with a sequence of Propionibacterium freudenreichii **transcarboxylase** 1.3S biotin subunit
AU Yamano, Naoko; Kawata, Yoshikazu; Kojima, Hiroyuki; Yoda, Koji; Yamasaki, Makari
CS Gov. Ind. Res. Inst., Osaka, Ikeda, 563, Japan
SO Bioscience, Biotechnology, and Biochemistry (1992), 56(7), 1017-26
CODEN: BBBIEJ; ISSN: 0916-8451
DT Journal
LA English
AB Biotinylation of fusion proteins in E. coli was studied using a sequence of Propionibacterium freudenreichii transcarboxylase 1.3S biotin subunit. As the biotinylation sequence, the authors examd. two sequences: one was of amino acid residues [84-123] of 1.3S, a partial sequence contg. a region from a conserved tetrapeptide (Ala-Met-Bct-Met) around the biotinyl lysine (Bct) to the carboxyl terminal; the other was of an almost entire sequence [18-123]. The authors constructed recombinant plasmids for fusion proteins of .beta.-galactosidase, of chloramphenicol acetyltransferase, and of alk. phosphatase. The authors found the biotinylation in the [18-123] sequence fused to alk. phosphatase.

L7 ANSWER 8 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1993:208135 HCAPLUS
DN 118:208135
TI The nonbiotinylated form of the 1.3 S subunit of **transcarboxylase** binds to avidin (monomeric)-agarose: Purification and separation from the biotinylated 1.3 S subunit
AU Shenoy, Bhami C.; Magner, William J.; Kumar, Ganesh K.; Phillips, Nelson F. B.; Haase, F. Carl; Samols, David
CS Dep. Biochem., Case West. Reserve Univ., Cleveland, OH, 44106-4935, USA

SO Protein Expression and Purification (1993), 4(1), 85-94
CODEN: PEXPEJ; ISSN: 1046-5928
DT Journal
LA English
AB Avidin-biotin technol. is used routinely to purify biotin-contg. carboxylases and also proteins that have been chem. coupled to biotin. The 1.3 S subunit of transcarboxylase (TC) studied here is the biotin-contg. subunit of TC which not only acts as a carboxyl carrier between the CoA ester sites on the central 12 S subunit of TC and keto acid sites on the outer 5 S subunit of TC but also links the 12 S and 5 S subunits together to form a 26 S multisubunit TC complex. The 1.3 S subunit has been cloned, sequenced, and expressed in Escherichia coli. A method for purifying recombinant 1.3 S subunits from E. coli using avidin (monomeric)-agarose column chromatog. has been developed. This affinity-purified 1.3 S was found to be homogeneous by SDS-PAGE, amino acid compn., and N-terminal sequence anal. but had a biotin content of only 28% based on moles of biotin per mol of 1.3 S. This lack of stoichiometry was due to copurifn. of apo-1.3 S as evidenced by the holocarboxylase synthetase reaction. A procedure for sepg. the apo- and biotinylated 1.3 S forms using hydrophobic interaction chromatog. on an Ether 5 PW column is described. The method is based on the difference in hydrophobicity between apo and biotinylated 1.3 S forms. The copurifn. of apo and biotinylated forms of 1.3 S on the avidin (monomeric)-agarose column was found to be due specific interaction with avidin rather than to interaction between apo- and biotinylated 1.3 S forms as demonstrated by the fluorescence quenching studies. The results suggest that the avidin-biotin system by itself may not be sufficient to obtain homogeneous biotinyl proteins as nonbiotinyl protein can also bind avidly to such columns.

L7 ANSWER 9 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1993:118267 HCAPLUS
DN 118:118267
TI Use of an avidin-binding polypeptide for affinity purification of proteins from transgenic hosts
IN Cress, Dean Ervin; Haase, Ferdinand Carl
PA Rohm and Haas Co., USA
SO Eur. Pat. Appl., 39 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 511747	A1	19921104	EP 1992-303067	19920407
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, PT, SE				
	US 6072039	A	20000606	US 1991-687819	19910419
	CA 2064933	AA	19921020	CA 1992-2064933	19920402
	AU 9213987	A1	19921022	AU 1992-13987	19920402
	AU 659139	B2	19950511		
	NO 9201364	A	19921020	NO 1992-1364	19920408
	BR 9201437	A	19921201	BR 1992-1437	19920416
	JP 06166698	A2	19940614	JP 1992-98307	19920417
PRAI	US 1991-687819		19910419		

AB A peptide that is a substrate for the enzyme biotin holoenzyme synthetase is used in fusion proteins to introduce a site for enzymic biotinylation. The biotinylated protein is then isolated by biotin affinity chromatog. under conditions that avoid the use of denaturants. The peptide is removed from the protein by specific proteolytic or chem. cleavage. An N-terminal domain from the 1.3S subunit of the transcarboxylase of

Propionibacterium shermanii was used as the biotin acceptor of a fusion protein. A synthetic gene for a .beta.-endorphin was placed 3' of the sequence encoding the biotinylation domain with the construct connecting the two domains via a labile methionine and the construct expressed in Escherichia coli from the tac promoter. The protein was rapidly purified from cell lysates by chromatog. on an avidin affinity column using acetic acid as the eluant. The fusion protein was cleaved with formic acid to yield .beta.-endorphin or the reverse product depending upon the orientation of the endorphin coding sequence.

L7 ANSWER 10 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1993:1991 HCAPLUS
DN 118:1991
TI **Biotinylation** of **proteins** synthesized in an heterologous host
IN Yamano, Naoko; Kojima, Hiroyuki
PA Agency of Industrial Sciences and Technology, Japan
SO Jpn. Kokai Tokkyo Koho, 4 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 04267880	A2	19920924	JP 1991-60953	19910220
PRAI JP 1991-60953		19910220		

AB A method for biotinylation of proteins manufd. by expression of the gene in an heterologous host is decribed. The method uses the biotinylation domain of the 1.3S subunit of the transcarboxylase of Propionibacterium freudenreichii as the biotin acceptor. Plasmid pHSGAPPT contg. the gene for alk. phosphatase (phoA gene) ligated in frame to the transcarboxylase subunit gene was prepd. Escherichia coli transformed with the plasmid produced the biotinylated alk. phosphatase with a mol. wt. of 57,500.

L7 ANSWER 11 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1991:443476 HCAPLUS
DN 115:43476
TI Fusion proteins having an in vivo post-translational modification site and methods of manufacture and purification
IN Cronan, John E., Jr.
PA Biotechnology Research and Development Corp., Inc., USA; University of Illinois
SO PCT Int. Appl., 120 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9014431	A1	19901129	WO 1990-US2852	19900517
W: AU, CA, JP, KR				
RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE				
CA 2057908	AA	19901120	CA 1990-2057908	19900517
AU 9058270	A1	19901218	AU 1990-58270	19900517
AU 647025	B2	19940317		
EP 472658	A1	19920304	EP 1990-909093	19900517
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
JP 04507341	T2	19921224	JP 1990-508763	19900517
US 5252466	A	19931012	US 1990-525568	19900518
PRAI US 1989-354266		19890519		

WO 1990-US2852 19900517

AB Recombinant proteins contg. a posttranslational modification site, i.e. a site for biotinylation or lipoylation, are prepd. The posttranslational modification aids in purifn. of the fusion proteins. Plasmids contg. chimeric genes for .beta.-galactosidase fused to biotinylation sites of Escherichia coli biotin carboxyl carrier protein, the 1.3S subunit of Propionibacterium shermanii transcarboxylase or a tomato sequence homologous to this protein, human pyruvate carboxylase, or Saccharomyces cerevisiae pyruvate carboxylase were prepd. E. coli transformed with these plasmids produced biotinylated fusion proteins which were purified on low-affinity "monomer avidin" columns (Sigma Chem. Co.). Fusion proteins contg. lipoylation site(s) of the E2p subunit of E. coli pyruvate dehydrogenase were similarly produced and purified with a p-aminophenylarsine oxide-Sepharose column.

L7 ANSWER 12 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1989:626534 HCAPLUS
DN 111:226534
TI Expression of synthetic genes fused to biotinyl region of **transcarboxylase** of Propionibacterium shermanii in Escherichia coli. Attempt of in vivo **biotinylation** to facilitate **protein** purification
AU Sato, Naoko; Kojima, Hiroyuki
CS Govern Ind. Res. Inst., Osaka, Japan
SO Osaka Kogyo Gijutsu Shikensho Kiho (1989), 40(2), 76-86
CODEN: OKGKAE; ISSN: 0472-142X
DT Journal
LA Japanese
AB In vivo biotinylation with synthetic genes was studied in order to facilitate purifn. of a recombinant gene product, based on specific affinity of biotin to avidin. A partial DNA sequence of Propionibacterium shermanii transcarboxylase 1.3 S biotinyl subunit was chosen for the biotinylation (biotin-tail, BT), including an evolutionarily conserved structure of biotin enzymes from the tetrapeptides of biotinylation site to the carboxyl terminal. Three expression vectors were constructed: the vector pDR-BT was directed to express only BT protein, pUC-BT to express a fusion protein of a part of .beta.-galactosidase and BT, and pDRCm-BT to express a fusion protein of chloramphenicol acetyltransferase and BT. Their expression products in Escherichia coli were analyzed by SDS-PAGE and fluorog. with 14C-biotin. No vector produced a biotinylated protein, although pUC-BT and pDRCm-BT produced fused proteins as expected. The failure of biotinylation is discussed.

L7 ANSWER 13 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1988:467890 HCAPLUS
DN 109:67890
TI Expression of the biotin biosynthetic operon of Escherichia coli is regulated by the rate of protein biotination
AU Cronan, John E., Jr.
CS Dep. Microbiol., Univ. Illinois, Urbana, IL, 61801, USA
SO Journal of Biological Chemistry (1988), 263(21), 10332-6
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB In E. coli biotin biosynthesis is repressed by high concns. of exogenous biotin. This paper reports that upon high level prodn. of the apo form of a biotinated protein, biotin operon expression was derepressed by 8-10-fold. The biotinated protein studied was the 1.3 S subunit of Propionibacterium shermanii, and transcarboxylase derepression was assayed by .beta.-galactosidase prodn. in strains which carry a lacZ gene altered

such that it is transcribed from biotin operon promoters. Depression of .beta.-galactosidase synthesis upon prodn. of the apo 1.3 S protein was obsd. over a several hundred-fold range of biotin concns. and also resulted in an increased level of biotin operon expression at maximally repressing biotin concns. Biotin operon derepression by apobiotin protein prodn. seems a direct consequence of the properties of the biotin repressor protein which also functions as the ligase catalyzing the covalent attachment of biotin to apoproteins.

L7 ANSWER 14 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1982:611487 HCAPLUS
DN 97:211487
TI The amino acid sequences of the biotinyl subunit essential for the association of **transcarboxylase**
AU Kumar, Ganesh K.; Bahler, Chris R.; Wood, Harland G.; Merrifield, Robert B.
CS Sch. Med., Case West. Reserve Univ., Cleveland, OH, 44106, USA
SO Journal of Biological Chemistry (1982), 257(22), 13828-34
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Transcarboxylase consists of a central subunit to which 6 outside subunits are linked by 2 biotinyl subunits/outside subunit. It has been shown previously that residues 1-42 of this sequence are sufficient to cause binding of the outside subunits to the central subunit. This investigation was undertaken to det. which portion of this sequence binds to the central subunit and which to the outside subunit. Sequence 1-14 was synthesized chem. and sequence 2-26 was obtained by CNBr cleavage of the biotinyl subunit. By ultracentrifugation, glycerol gradient centrifugation, and electron microscopy, it was shown that peptide 2-26 binds the outside subunits to the central subunit, but peptide 1-14 does not. There is an enhancement of fluorescence when the peptides bind to the subunits. With peptide 2-26, the enhancement occurred with either the outside subunit or the central subunit, but, with peptide 1-14, it occurred only with the central subunit. Binding of the peptides was also tested by detg. their effect on formation of enzymically active transcarboxylase from the native subunits. Peptide 2-26 was shown to inhibit combination of the biotinyl subunit with the outside subunit and thus retard combination with the central subunit and to cause a decrease in the enzymic activity when compared with untreated subunits. Peptide 1-14 had no effect, showing it did not compete for the binding site on the outside subunit. Where the 12 SH subunit was treated with either peptide, there was inhibition of formation of active enzyme, showing that each bound to the site on the central subunit. Apparently, of sequence 2-26, 2-14 binds to the central subunit and 15-26 to the outside subunit or portions thereof. It is proposed that some residues in the central portion of sequence 2-26 are not bound to either the central or outside subunits and that the variation in distance which is obsd. in electron micrographs of transcarboxylase between the central and outside subunits results from this flexible portion of the sequence.

L7 ANSWER 15 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1980:2376 HCAPLUS
DN 92:2376
TI Amino acid sequence of the biotinyl subunit from **transcarboxylase**
AU Maloy, W. Lee; Bowien, Botho U.; Zwolinski, Gene K.; Kumar, K. Ganesh; Wood, Harland G.; Ericsson, Lowell H.; Walsh, Kenneth A.
CS Sch. Med., Case West. Reserve Univ., Cleveland, OH, 44106, USA
SO Journal of Biological Chemistry (1979), 254(22), 11615-22
CODEN: JBCHA3; ISSN: 0021-9258

DT Journal
LA English
AB The complete amino acid sequence of the biotinyl subunit of transcarboxylase of Propionibacterium shermanii was detd. from the structures of overlapping tryptic and CNBr peptides together with sequenator anal. on the whole subunit. The subunit contained 123 amino acid residues. Eleven of 19 residues in the region of biotin attachment, when compared to pyruvate carboxylase from avian liver, were in identical positions relative to biocytin (Bct). There was less homol. with acetyl-CoA carboxylase from Escherichia coli, but in all of these biotin enzymes there was an Ala-Met-Bct-Met sequence. The secondary structure of the biotinyl subunit was estd. and considered in relation to the role of the biotinyl subunit in the structure and function of transcarboxylase.

L7 ANSWER 16 OF 17 HCAPLUS COPYRIGHT 2003 ACS
AN 1975:166689 HCAPLUS
DN 82:166689
TI Isolation of **peptides** from the carboxyl carrier subunit of **transcarboxylase**. Role of the non-**biotinyl peptide** in assembly
AU Ahmad, Fatzal; Jacobson, Birgit; Chuang, Margaret; Brattin, William; Wood, Harland G.
CS Sch. Med., Case West. Reserve Univ., Cleveland, OH, USA
SO Biochemistry (1975), 14(8), 1606-11
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
AB The carboxyl carrier subunit of transcarboxylase (EC 2.1.3.1) was required for assembly of the 12 S and 5 S subunits into the oligomer. However, only a portion of the subunit was required for this assembly. On treatment of transcarboxylase briefly with trypsin at pH 6.3, extremely susceptible peptide bonds of the carboxyl carrier protein were cleaved releasing biotinyl peptides of .apprx.66 and .apprx.40 residues. The resulting trypsinized transcarboxylase, though enzymically inactive, remained essentially intact as judged by its hydrodynamic and mol. sieving properties. The residual portion of the carboxyl carrier protein (nonbiotinyl peptide) was isolated by dissocn. of the 5 S subunit complex at pH 9 and by chromatog. over Bio-Gel A-1.5m. The isolated nonbiotinyl peptide contained the combining domain of the 1.3 SE carboxyl carrier protein since it caused combination of the 12 S and 5 S subunits. The .apprx.66- and .apprx.40-residue biotinyl peptides, released by the trypsin treatment, apparently occur on an exposed portion of the enzyme. This portion of the carboxyl carrier protein apparently serves to place the biotinyl group adjacent to the 2 substrate sites of the enzyme, 1 of which is on the peripheral subunit and the other on the central subunit. Thus, the carboxyl carrier protein has 2 functions: 1 portion holds the 12 S and 5 S subunits in juxtaposition and the other portion orients the biotinyl group adjacent to the substrate sites so that it may function as a carboxyl carrier between the sites.

L7 ANSWER 17 OF 17 WPIDS (C) 2003 THOMSON DERWENT
AN 2003-165810 [16] WPIDS
DNN N2003-130921 DNC C2003-042975
TI Novel fusion **protein** useful for targeting desired **protein** to cell in culture or in the body of subject, comprises **biotinylation**-competent **protein/peptide**, or **biotin** acceptor **peptide** (BAP), and desired polypeptide.
DC B04 D16 S03
IN BARRY, M A; PARROTT, M B
PA (BAYU) BAYLOR COLLEGE MEDICINE

CYC 1
PI US 2002142355 A1 20021003 (200316)* 13p
ADT US 2002142355 A1 Provisional US 2000-247965P 20001114, US 2001-987485 20011114
PRAI US 2000-247965P 20001114; US 2001-987485 20011114
AB US2002142355 A UPAB: 20030307
NOVELTY - A fusion **protein** (I) consisting essentially of a **biotinylation**-competent **protein** or **peptide**, or a **biotin** acceptor **peptide** (BAP), and a polypeptide of interest, where the **biotinylation**-competent **protein** or **peptide**, or BAP is joined directly to the N- or C-terminal end of the polypeptide of interest, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a polynucleotide vector (II) for expressing **protein** comprising a coding region consisting of nucleotides encoding (I), and a promoter active in mammalian cells and operably linked to the coding region; and

(2) **biotin**-labeling (M) a virus, involves replicating the virus in a mammalian host cell, where the host cell expresses a **biotin** ligase and has been engineered to express (II).

USE - (I) is useful for targeting a **protein** of interest which is on the surface of a virus, to a cell in culture in the body of a subject. The method involves binding avidin to the surface of the cell, **biotinylating** (I), where the **protein** of interest is joined to the **biotinylation**-competent **protein** or **peptide**, and administering the **biotinylated protein** to either the medium surrounding the cell in culture or to the subject. The avidin is bound to the surface of the cell by attaching avidin to a ligand that binds to a receptor located on the surface of the cell, and administering the avidin/ligand molecule either to the medium surrounding the cell in culture or to the subject. The **protein** of interest is used to target the virus to the cell. (II) is useful for **biotinylating** a polypeptide of interest secreted by a mammalian host cell, by expressing (II) in a mammalian host cell in vivo or in vitro. The cell is a Chinese hamster ovary (CHO) cell in culture, and is engineered to express a distinct fusion **protein** consisting of a **biotin** ligase (e.g. BirA) directly linked to a leader sequence (e.g. Igkappa secretory leader) that promotes secretion from the host cell (all claimed).

(I) is useful for drug and gene therapy targeting. The **biotin** labeled **proteins** are useful for delivering nucleic acids to cell in vivo. (M) is useful for rapidly purifying virus, for attaching other compounds to the virus, for modifying the virus's ability to transduce cells in vivo and ex vivo, and for directing the virus to specific avidin-tagged sites in a patient's body.

ADVANTAGE - (II) effectively labels polypeptides with **biotin**

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